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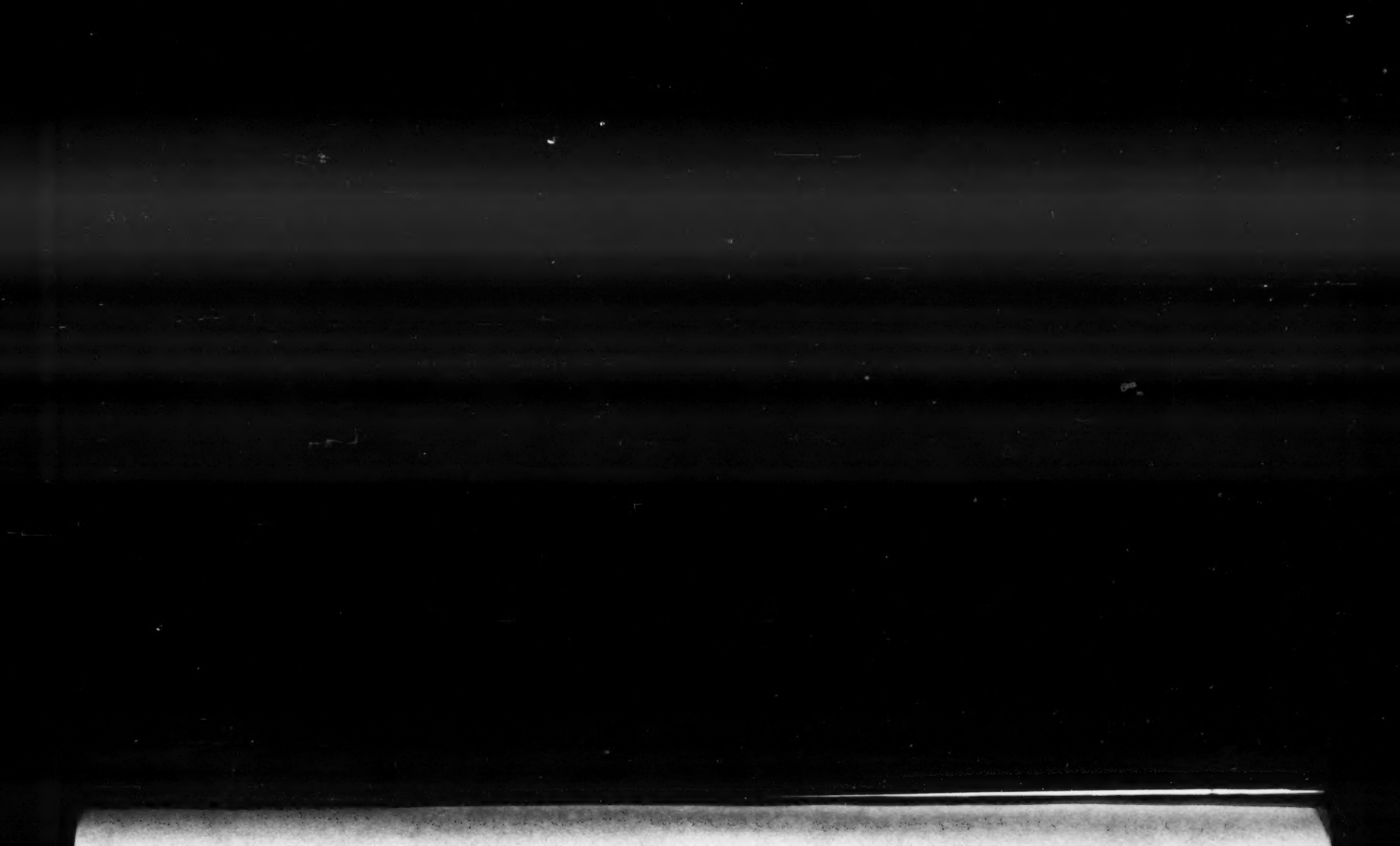

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PLANT PHYSIOLOGY

OCTOBER, 1930

THE FEEDING POWER OF PLANTS*

WALTER THOMAS

A. Introductory

1. VARIABLE COMPOSITION OF IDENTICAL SPECIES

The publication of WOLFF's monumental work on "Aschen-analysen" in 1871 (**210**), although compiled principally for the purpose of supplying information on the composition of plants used in feeding farm animals, nevertheless, served to crystallize the large amount of experimental data of English, French and German investigators that had accumulated even in those early days of agricultural science. WOLFF's data showed very clearly that plants even of the *same species* differed considerably both in the *absolute* and *percentage amounts* of the various elements absorbed, depending upon the composition and nature of the substrate (soil or nutrient solution). Indeed, WOLFF himself (**209**) was the first to give definite proof that the composition of the ash of any plant could be changed by varying the proportion of salts in the nutrient culture medium.

2. SELECTIVE POWER OF DIFFERENT SPECIES

Numerous investigations, those of NEWTON (**129**) being the most recent, have since shown, moreover, that *different species* growing in media of identical composition possess selective powers with respect to any specific ion or ions. This marked difference in the assimilatory powers of different plants is very clearly exhibited in their different responses to such "insoluble" mineral fertilizers like rock phosphate, basalt, gneiss, etc.

3. ARE DIFFERENCES IN RESPONSE A SPECIES CHARACTERISTIC

But as the environmental conditions even in the water and sand culture experiments cited by WOLFF were never identical, the question as to the

* Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as technical paper no. 502.

nature of the factors operative was still left open. Nor was it decided whether the differences in responses exhibited were characteristic of the species or the result of environmental or other unknown factors. And, although NEWTON (**126**) believes that with the exception of phosphorus (**129**) there is no characteristic difference in the *type of absorption* of certain species of plants (barley and peas, for example) in nutrient culture solutions, *i.e.*, that there is no difference in percentage content with respect to any element in the plants examined that holds consistently in favor of one or the other throughout all stages of growth, it will be apparent as the subject is developed that the evidence is overwhelming that different plant species growing in media of identical composition not only exhibit differences in composition that can only be interpreted to indicate marked differences in the type of absorption (**5**) but that they exhibit also differences in what has been frequently designated in the literature by the expression feeding power or ability to utilize insoluble soil constituents to different degrees, which are characteristic of the species. The following data from KÖNIG's experiments (**82**)—which are among the earliest—are typical as exemplifying this point:

TABLE I

PER CENT. OF K_2O IN OATS AND PEAS GROWN UNDER SIMILAR CONDITIONS

	SANDY	LOAM	LOAM	CALCAREOUS	CLAY
Yields (first year) Oats	0.396	0.506	0.420	0.489	0.401
Peas	0.554	0.688	0.554	0.666	0.702

4. THE TERM "FEEDING POWER" DEFINED

The term "absorbing power" or "feeding power" as it is known in technical agronomy may connote several ideas (**67**). Many have objected to the use of the term feeding power. SHULL (**172**) suggests that some plant physiologists prefer not to use the term on the basis that it has too much psychological implication and others (**67**) because of the many different interpretations given to it. But, as CROCKER (**27**) points out, although the expression has undesirable implications, it is better to continue its use than to invent some new phrase that might, perhaps, be freer from these implications, because other terms suggested, like "absorbing power of crop plants" or even "specific absorbing power," do not imply quite enough. The term "feeding power" implies both power to absorb the soil nutrients and also the power of making use of many of the salts absorbed. This expression will be used in the present paper as a concise means of indicating

the difference exhibited by different types of plants in the *absorption* and *utilization* of difficultly available minerals, either native to the soil or added as soil amendments, as determined by the differences in the amount of nutrient elements absorbed from the minerals in question, the utilization of which is generally exhibited in different growth responses.

5. Theories advanced to account for different responses

From the numerous experiments conducted to determine the cause of the characteristic differences in absorbing power by different species, a number of explanations have been advanced. The differences have been attributed to (1) the differences in the chemical nature (and possibly amount also) of other products of root exudations (acids, carbohydrates, etc.); (2) the differences in (a) the absolute amounts of carbon dioxide evolved, or (b) the amounts evolved per gram of dry weight of the roots (respiration energy); (3) the operation of the law of mass action between the carbon dioxide (carbonic acid) from the roots and the "insoluble material" in the soil; (4) unlike potential differences between the soil and plant; (5) differences in the permeability of the root membranes producing "selectivity" in absorption of roots; (6) the existence of a Donnan membrane equilibrium, and to a combination of one or more of the above factors (**67**).

We shall discuss the evidence for the operation for these various factors separately.

B. The chemical nature of the root exudations

1. The carbon dioxide factor

The problem of determining the nature of root secretion is of importance not only from the standpoint of scientific inquiry, involving the fundamental question of the mechanism of absorption of nutrients and the reaction of protoplasm, but is of importance also from the practical aspect with respect to the economic utilization of minerals occurring naturally in the soil, and applied also as soil amendments.

a. Considerations of some of the experimental difficulties involved.—It must be granted that even if proof were forthcoming that carbon dioxide is the only factor involved, as indeed Hall (**55**) points out, the problem of isolating the factors that contribute to the sum total of the soil carbon dioxide is not a simple one. In the first place, the carbon dioxide is not all in the gaseous phase, a large part (**163**) (and, indeed, under some conditions nearly all) is present in solution (**93**); and in the second place, the soil respiration is the total of all the soil processes that give rise to the production of CO_2, *viz.*, oxidation (**83, 169, 213**), the metabolic processes of the microorganisms (**110, 181, 182, 183, 213**), and the

respiration of roots and of the bacteria that inhabit them (**58, 63, 110, 178, 181, 208**). It follows that the concentration of carbon dioxide, as measured in most experiments conducted with soils, is a function of the absolute CO_2 produced by the soil particles together with the roots, and also of the diffusion velocity (**102, 103**).

Since the distribution of the CO_2 between the gaseous and liquid phases will depend upon a number of variables (water and calcium principally), the net effect of any one factor at any time is necessarily difficult to determine. RUSSELL (**162**) believes that the CO_2 evolved from plant roots cannot be distinguished from that given off by microorganisms and this is also the conclusion drawn by STARKEY (**177, 178**); but the work of STOKLASA (**182**), HEADDEN (**60**), TURPIN (**198**), BARAKOV (**3**) and NELLER (**124**) indicates that approximate relative differentiation is possible.

b. THE VIEWS OF THE SEVERAL SCHOOLS.—Plant physiologists and soil scientists have been and still are divided into several schools. One group (**10, 155, 162**) maintains that plants do not dissolve *significant* quantities of minerals that would otherwise remain undissolved; another school emphasizes the important rôle that the root exudations exercise in this connection. The latter school is divided into two groups: One (**26, 38, 39, 49, 77, 89, 96, 141, 145**) subscribing to the view that "insoluble" substances are rendered soluble by the exudation from the roots of plants of substances (*e.g.*, organic acids, acid salts, etc.) other than carbon dioxide; and the other school (**1, 52, 55, 60, 117, 121, 182**) that only CO_2 is exuded from the roots and that the influence of plants on the solubility of soil minerals is to be attributed solely to this factor. The rôle played by microorganisms in the soil and especially the action of microorganisms of the rhizosphere, isolated by STOKLASA (**181**) is recognized by all schools.

c. CHARACTERIZATION OF OBJECT OF EXPERIMENTS IN THIS FIELD.—Consequently, in the examination of the available evidence for the purpose of evaluating the contribution of the carbon dioxide evolved from plant roots as a factor in dissolving the "insoluble" soil materials, we must distinguish between (1) those experiments (**3, 31, 59, 60, 84, 85, 86, 92, 102, 103, 163, 182, 198, 211, 212**) that have for their purpose the determination of the relative contribution of carbon dioxide by root and microorganisms of the soil, and (2) those (**28, 38, 49, 77, 89, 117, 121, 145**) which, although accepting the important rôle of the carbon dioxide evolved from the roots, were carried out for the purpose of determining if acids and other substances than CO_2 are exuded by roots, and (3) those (**37, 139, 140, 181, 191**) that are concerned with the effect of CO_2 from whatever source derived.

d. OBSERVATIONS OF THE EARLIER INVESTIGATORS.—The older "corrosion" experiments of SACHS (**165**), KNOP (**80**), LIEBIG (**97**), KNY (**81**),

CZAPEK (**28, 29**) and MOLISCH (**122**) and observations of the natural etchings frequently found upon calcareous stones lying in the soil were regarded as furnishing *prima facie* evidence that the roots of plants participate in dissolving "insoluble" nutrients of the soil. And more recently FRED and HAAS (**44**) found that this action of roots on marble is increased when a sterilized soil was inoculated with pure cultures of various bacteria.

The growth fusion ideas (**26, 176**) and etching experiments of SACHS (**165**) and of CZAPEK (**29**) are of interest here. The former observed that only the smooth surfaces of the minerals that were soluble in carbonic acid showed "corrosion figures"; silicates did not. CZAPEK (**29**) sought further information on the problem by substituting plaster of Paris slabs which were incorporated with various carbonates and phosphates. He argued (**28, 29**) that since—in addition to the carbonates—the phosphates of calcium, magnesium and iron, but not of aluminum phosphate, were corroded by roots, the acids exuded from the roots must be limited to carbonic, acetic, propionic and butyric. Finally, all but carbonic acid were eliminated by means of qualitative results with Congo red. CZAPEK's deduction from these experiments, however, is not valid, for the evidence from the experiments of CAMERON and HURST (**20**), PRIANISHNIKOV (**151, 153, 155**) and MARIAS (**107**) is conclusive that aluminum phosphate is available to plants through hydrolytic processes. The use, therefore, of $AlPO_4$ is not justifiable in experiments to decide the nature of root exudations.

Moreover, all these "corrosion" experiments may be criticized on the basis that no mineral phosphates exist that are unavailable to plants when all factors that hinder the dissolving power of the roots are removed. The experiments of BUTKEWITSCH (**16**), which will be discussed later in this paper, exemplify this very clearly.

e. THE USE OF SEEDLINGS IN DETERMINING THE NATURE OF ROOT EXUDATIONS.—The approach to the problem by the use of seedling plants, moreover, is not sound (**1, 28, 29, 52**). The mineral requirements of such young plants are too small to justify the suitability of such experiments to answer the question of root exudations, by whatever method and however carefully carried out. The conclusions from such experiments must be of doubtful value. For this reason we shall consider only those experiments in which mature plants were used.

f. THE PROBLEM OF INTERPRETATION.—The results of field plot experiments (**59, 60, 92, 102, 103, 163, 211**) are contradictory, but pot and lysimeter experiments (**3, 92, 128, 182, 198**), with certain exceptions (**86**) where the technique adopted may be open to criticism (**55**), agree in attributing to the roots a considerable and, indeed, preponderant contribution to the carbon dioxide of the soil.

(1) THE WORK OF THE ROTHAMSTED GROUP.—Notwithstanding the fact that on the Broadbalk field and Hoos plots RUSSELL and APPLEYARD (**163, 164**) found, in agreement with other investigators (**3, 9, 59, 60, 92, 198**), that at certain seasons of the year, especially at the time of active growth and ripening, cropped soils contain a higher content of CO_2 than uncropped soils, their interpretation of the results differ. RUSSELL and APPLEYARD (**163**) attribute the conflicting results of the older workers (**40, 41, 123, 211**), even when comparison was made between cropped and fallow portions of the same plot, to failure to recognize the marked influence of differences in the physical and chemical structure on the carbon dioxide content of the soils in question. The Rothamsted investigators conclude that in their own experiments the difference in the physical structure and composition of the cropped (or grass) and uncropped (fallow land) soils makes comparison difficult. These authorities observed that the smaller the soil differences become, the smaller is the effect of the crop on the production of CO_2 and they postulate that, if it were possible to obtain absolute identity of conditions, the effect of the crop would vanish. But that the factor assigned by these authorities for the observed differences cannot be the only one is shown by the work of HEADDEN (**60**), who found a marked depression of soil carbon dioxide after each cutting of alfalfa. There is, moreover, such a marked difference in the amount of CO_2 evolved from an uncropped and a cropped plot in some of the more recent field experiments (**59**) that it is difficult to conceive how such marked divergence could be due to physical differences in the soil of the plots. For example, HASSE and KIRCHMEYER'S (**59**) results indicate that four-fifths of the total CO_2 was due to root respiration.

(2) ARE LYSIMETER RESULTS APPLICABLE TO FIELD CONDITIONS?—Moreover, if RUSSELL and APPLEYARD'S (**163**) conclusions are generally applicable, the question may be raised whether pot and lysimeter experiments of the type conducted by BARAKOV (**3**), TURPIN (**198**) and others (**9, 37**) are applicable to field conditions. That the deductions drawn from some of these experiments may not be applicable to field conditions, on account of the conditions being too artificial, is possible. This criticism would apply to the conclusions of KOSSOWITSCH (**84, 85, 86**) in which CO_2 was determined in percolates of the nutrient solution in quartz sand cultures, but for the fact that DUSTMAN (**37**) has shown that, although the absolute respiration of plants grown in quartz and soil cultures is greater in the former, the relative differences between different plants are the same in both.

In TURPIN'S lysimeter experiments (**198**) the difference between the amount of carbon dioxide in the cropped soil and that in the uncropped

soil at the *period of most active crop growth,* divided by the amount of water transpired by the crop, gave a constant which varied with the season. Moreover, as there was no indication that the increased CO_2 in the cropped soil arose from the decomposition of root particles, TURPIN logically concludes from these experiments that the plant often produces at the period of its most active growth many times as much CO_2 as is produced by soil organisms.

The results of BARAKOV's lysimeter experiments (**3**), in which weekly determinations of CO_2 were made on different types of unsterilized soils, also indicate that the principal source of CO_2 is from the respiration of roots. The greatest absolute quantity of CO_2 was associated with the greatest development of the plant. He notes that each plant experimented with had its own specific respiration curve.

STARKEY (**177, 178**), however, frequently found little effect on the CO_2 content of the soil or abundance of organisms, especially in the early stages of growth. He also noted the occurrence of larger numbers of microorganisms about plant roots than at a distance from them. On the basis of these findings STARKEY questions the conclusions of TURPIN (**198**) and BARAKOV (**3**). There is no reason to doubt that at certain periods a part of the carbon dioxide evolved in the respiration of roots is contributed by the bacteria that inhabit the root surface, *i.e.*, the rhizosphere (**58, 110, 180**); but the weight of evidence (**3, 60, 198**) indicates that the total contribution to the soil CO_2 by such means is relatively insignificant compared with the amount evolved during the growing season by the respiratory activity of the roots. It is also apparent on physico-chemical grounds that determinations of carbon dioxide on samples of soil taken under such conditions as those described by STARKEY (**177**) afford no basis for drawing conclusions concerning the quantitative relations relative to the carbon dioxide of the soil atmosphere *in situ*. This same disadvantage applies also to METZGER'S (**111, 112**) attempt to measure the concentration of bicarbonates in soil samples immediately around the roots with those taken away from the roots, for these methods obviously destroy the CO_2 equilibrium conditions actually existing in the soil. A review of methods used for the determination of carbon dioxide of the soil atmosphere will be found in a paper by POTTER and SNYDER (**150**)

HUTCHINSON (**72**) concludes that there exists a parallelism between bacterial numbers and CO_2 content of the soil. This report (**72**) is frequently cited in the literature; but it is sketchy and lacking in details. If the exact parallelism between soil CO_2 and bacterial numbers occurs throughout the growing season, this fact would tend to show that the root contribution would be small; but when such correlations are attempted

(**72, 163**) there are well defined periods when the bacterial numbers fall as the CO_2 increases. If, then, as WOLLNY and others (**144, 198, 213**) suggest, relatively large increases of CO_2 depress bacterial activity, no correlation would be expected, which may, as PLUMMER (**149**) suggests, be due to the limitation of the oxygen supply. It should be noted, however, that STOKLAS (**180**) finds that root respiration and the number of bacteria in the rhizosphere paralleled one another.

We must, therefore, conclude that either RUSSELL and APPLEYARD'S (**163**) results were due to conditions not readily duplicated and that the conclusions drawn by them are not of general application, or that deductions from experiments of this type carried out in pots and lysimeters are not applicable to conditions in the field. The assimilation and utilization of nutrients in pot experiments is considerably greater than in field experiments. In the former the roots penetrate the soil more intensively than in the open field. As a result of this better utilization of nutrients, the yields in pot experiments are frequently six to ten times higher than those in the field. Recent suggestions (**32**) to eliminate these differences include mixing the soil with equal parts *by volume* of quartz sand. Finally, it is to be noted that there is no evidence that the discrepancies in the experiments discussed above can be attributed to the relative predominance of root respiratory activities as a result of the relatively low rate of oxidation of organic matter or *vice versa*.

g. INTENSITY OF CO_2 PRODUCTION BY ROOTS OF DIFFERENT SPECIES.—Since the atomic groupings and reciprocal linkages of the roots of different plants are not similar, it could be postulated that the respiration activity of the root systems of plants would be different. The complexity of the problem is appreciated when specific data for the respiratory energy of the roots of different plants are considered. The causes of the wide variations reported by different investigators for identical types of plants must be sought in the different conditions of experimental technique, especially with respect to the difficulty in making a comparison of the influence of the various factors that influence respiration, such as concentration of oxygen, temperature, concentration of carbon dioxide, and nature and concentration of nutrients.

Thus, the average amount of CO_2 evolved in 24 hours from barley grown to maturity was found by STOKLASA and ERNEST (**182, 183**) to be 70 mg. per gm. dry weight of roots, whereas NEWTON (**127**), in plants 35 days old, found only 3.5 mg. of CO_2 per gm. dry weight of roots evolved in 26 hours. Both of the above investigators used sand as the substrate. STOKLASA used sterilized sand and KNOP'S nutrient solution. NEWTON used HOAGLAND'S culture solution. NEWTON has thought that possibly his method

of simple diffusion may not have measured all the CO_2 evolved (**130**). But the agreement between DUSTMAN'S (**37**) results and NEWTON'S shows that this is not the reason and, theoretically, no great difference would be expected to result between systems depending on diffusion and air currents, respectively, since the measurements must necessarily have been made when each system was in a state of equilibrium between output and removal of CO_2 (**104**); nor is there any evidence that these large differences in STOKLASA'S and NEWTON'S values can be attributed to any great difference in the age of the plants used. Values obtained by the writer (**186**) for sweet clover in sand cultures by a similar method to that used by PFEIFFER and BLANCK (**145**) ranged from 8–9 mg. per gram. of dry weight of roots evolved in 24 hours, compared with values of 4.5 mg. obtained by NEWTON. DUSTMAN'S (**37**) results are in relative agreement with NEWTON'S. That STOKLASA and ERNEST'S (**182**) values are far higher than is usual under field conditions will be evident from a consideration of the following facts: STOKLASA concludes that the microorganisms in an acre of soil to a depth of 1.5 feet may produce between 65 and 70 pounds of CO_2 a day for 200 days in the year, and that during the growing period the roots of oats or wheat would give off nearly as much to an acre. This would mean a loss from decomposition of organic matter of 13,500 pounds of CO_2 per acre per year, or $0.471 \times 13{,}500 = 6{,}358$ pounds of organic matter. This value is abnormally high. As NEWTON (**130**) points out, a loss of one-half to one ton of organic matter per acre per year is closer to the average loss from normal soils.

It must be admitted (**87**) that calculation of the carbon dioxide production per gram of dry weight of material is not a very refined method of measurement, owing to the variation in the percentage amount of protoplasm in the various plants and parts of plants, the respiratory gas exchange of which depends entirely upon the activity of the living substance. Some tissues, such as wood and cork, consisting for the most part of dead cells, do not respire at all. But, owing to the impracticability of the alternative methods proposed (**87**), the simple method of calculation on the basis of total weight must be used.

Studied from a *relative standpoint*, however, STOKLASA and ERNEST'S results are decidedly valuable. Thus, the specific respiration energies (amount of CO_2 evolved per gram of dry weight) of roots found by them were barley 68, wheat 90, oats 127, rye 118, buckwheat 227. In sterilized sand culture experiments they found that the above values were in the same relative order as the utilization of P_2O_5 and K_2O from basalt and gneiss. As already pointed out, BARAKOV (**3**), too, in experiments with lupin, oats, barley and rye, found that the capacity to absorb Ca from rock

phosphate paralleled their values for the specific respiration energy. STOKLASA and ERNEST, whose results are in substantial accord with those of BARAKOV's (**3**), point out that the values found by them for the respiration energies do not, however, afford any indication of the relative yields under conditions where readily assimilable nutrients are supplied. Thus, in KNOP's nutrient solution the yield of barley was found to be far greater than that of wheat, rye or oats. This might be attributed to the compensating factor of extensive root development in the case of barley, as HESSE's measurements (**62**) of the root hairs of barley, oats, rye and wheat indicate and which are in harmony with the measurements of NOBBE (**131**).

TABLE II

SIZE OF ROOT HAIRS ON CEREAL CROP PLANTS

	Length	*Diameter*
	mm.	*mm.*
Barley	1.40	0.0102
Oats	0.98	0.0090
Rye	0.86	0.0070
Wheat	0.62	0.0068

If STOKLASA and ERNEST's results can be corroborated we could postulate: (1) That when the rate of supply of N, P and K are sufficiently rapid, as in the case of access to an ample supply of easily soluble nutrients, the yield will be proportional to the extent of root system. (2) But if one of these elements, such as phosphorus, is present only in a difficultly soluble form, the yield will be proportional to the respiration energies.

If these conclusions of STOKLASA and ERNEST (**182**) and of the other investigators cited (p. 446) can be proved to be of general applicability to our economic plants, we should be forced to accept the views of the school that regards the rôle played by carbon dioxide as the greatest factor determining their feeding power. The need, therefore, for more exact information on the respiration coefficients of the various types of economic plants is apparent.

2. TO WHAT EXTENT DOES CO_2 FUNCTION AS A SOLVENT

a. THE IMBIBITIONAL WATER OF THE ROOT HAIRS IS NOT A SATURATED SOLUTION OF CARBON DIOXIDE.—ABERSON (**1**), on the basis of titration and hydrogen-ion concentration values obtained on *seedling roots*, concluded that the hydrogen-ion concentration of the water of imbibition of the mucilaginous "membranes" of the root hairs in close contact with the soil particles contains a saturated solution of carbon dioxide. Experimen-

tal evidence is cited to show that a saturated solution of CO_2 is able to bring into solution the insoluble soil minerals, especially phosphates.

But ABERSON's view is not valid for the following reasons: (1) A saturated solution of carbon dioxide has a hydrogen-ion concentration of 1.2×10^{-4}; this exceeds the hydrogen-ion concentration values of any of the root exudations found by ABERSON. (2) With any given content of carbon dioxide in the soil atmosphere it is possible to calculate the content of CO_2 and HCO_3 ions in the water of imbibition in contact with it.

Since $HA = H^+ + A^-$ (1)

$$\frac{[H^+]\ [A^-]}{[HA]} = K_a \tag{2}$$

$$\therefore [H^+]^2 = K_a \cdot [HA] \text{ or } [H^+] = \sqrt{K_a \cdot [HA]} \tag{3}$$

and $$[H^+] = \sqrt{K_a \cdot (C - [H^+])} \tag{4}$$

where C is the concentration of the original acid.

Since $[H^+]$ is small, we have

$$[H^+] = \sqrt{K_a \cdot C} \tag{5}$$

or $pH = -1/2 \log. K_a + 1/2 \log C$ (6)

For polybasic acids the first ionization constant may be taken, since the second ionization constant is small compared with the first.

Since the HENRY coefficient at N.T.P. is approximately 1:1 (**199**), the percentage by volume of carbonic acid of the soil solution and the percentage in the soil air are approximately identical. Hence, even if the soil air contained as high as 10 per cent. CO_2, the soil solution (if it consisted of pure water) would also contain 10 per cent. by volume of CO_2, since the HENRY coefficient is 1:1. The pH of such a solution calculated from the above equation would be 4.10. Since, however, it is a solution of salts, and especially of bicarbonates, the dissociation of the carbon dioxide would be depressed even below the above value. Moreover, ABERSON has neglected to consider the influence of the salts present in the cell sap of the roots.

b. HOAGLAND'S VIEW.—The data of HOAGLAND *et al.* (**68, 70, 170**) would seem to afford evidence that CO_2 may not play an important rôle in *all* soils. These investigators found no measurable change in the reaction of soils of such markedly acid character of which the hydrogen-ion concentration is of the order of magnitude of the dissociation constant (3.0×10^{-7}) of carbonic acid (**200**), when such soils are partially saturated with carbon dioxide. In soils having a lower hydrogen-ion concentration (higher pH), however, the change in reaction due to partial saturation with CO_2 was

quite significant. The decomposition of stall or green manures in *acid* soils of hydrogen-ion concentration of the above order of magnitude would, therefore, not be expected to increase the hydrogen-ion concentration of the soil to such an appreciable extent as to have any effect on the solution of "insoluble" materials. Inasmuch as there is an overwhelming amount of evidence from field and laboratory experiments that decomposing matter is a potent source of rendering the "insoluble" minerals of the soil available, the rate of decomposition varying with the age and nature of the material (**108**) and also nature of fertilization (**206**), we should—on the basis of HOAGLAND and SHARP's data—be forced to attribute the major favorable effect in the majority of soils not to carbonic acid but to the intermediate products of decomposition by the soil microorganisms, such as butyric, acetic and lactic acids—the dissociation constants of which are of the order 1.8×10^{-4} to 1.7×10^{-5}—and possibly also to replacement effects in the exchange complex.

HOAGLAND (**68**) sums up the situation as follows: "It appears to me that some soils are so acid that CO_2 is of slight effect, and that in other soils the nature of the soil minerals is such that CO_2 may have very little influence in promoting the solution of PO_4 or K; but, nevertheless, that CO_2 is of great general significance because of the existence of a great number of soils—not inconceivably the majority of soils—where CO_2 does appreciably alter the culture medium of the plant. In all this, we presumably must constantly keep in mind the difficulty of knowing just what the chemical system, and influence of CO_2, is at root-soil particle interphases." Yet it is evident from more recent field experiments (**166**) that there are exceptions even to this generalization.

The influence of nitrification processes must, however, not be underestimated (**187**). In soils rich in calcium, nitrification processes are generally accompanied by the destruction of bicarbonates, *i.e.*, with a decrease in the buffer value and increased utilization (p. 466). In practice, then, the aim should be made to increase the decomposition of organic matter without greatly increasing the calcium concentration of the soil solution. One way of doing this would be to apply lime in small amounts.

c. EXPERIMENTS ON THE INFLUENCE OF ARTIFICIAL ADDITIONS OF CARBON DIOXIDE TO THE SOIL.—It might be expected that the results of experiments in which definite quantities of carbon dioxide are introduced into the soil in which plants are growing would give results from which definite conclusions could be drawn; but this is far from being the case. LUNDEGÅRDH (**103**) has shown the beneficial effects of increasing the carbon dioxide concentration of the atmosphere surrounding the external assimilatory organs, and BREAZEALE and BURGESS (**11**) noted increased absorption of

PO_4 by seedlings from "floats" when saturated with CO_2. The results obtained by MITSCHERLICH (**118**), PFEIFFER and BLANCK (**145**), and also by PARKER (**140**) are, however, decidedly negative with respect to its influence on the absorption of difficultly soluble materials; even distinct injury has resulted in some experiments (**118, 145**). These negative results, however, afford no proof that the carbon dioxide produced under field conditions does not play an important rôle in the assimilation of mineral nutrients. As TRUOG (**50**) suggests, the equilibrium conditions governing carbon dioxide evolution in the soil at the interface of the plasma membrane of the root hairs may not as yet have been duplicated in the laboratory.

It is unfortunate that all investigators have used either quartz sand or soils low in colloidal material in which the factor of ion replacement does not play the important rôle that it does in soils high in colloidal material. In view of the fact, moreover, that carbon dioxide is well known to have toxic effects when the CO_2/O_2 ratio is relatively high (**21, 24, 56, 118, 145, 167**), this possibility must also be considered in the experiments hitherto conducted on the effect of CO_2 additions. PFEIFFER and BLANCK (**145**) observed distinct injuries, especially to oats, in their first series of wheat, lupine and oats cultures, when washed CO_2 gas was introduced at the bottom of the vessel for 10 minutes, 3 days a week at a pressure of 2.5 atmospheres. In their second series, when water saturated with CO_2 was used, no injury resulted; but even this procedure did not in MITSCHERLICH'S experiments (**118**) prevent injury to the plants.

In none of the experiments on the effect of the artificial addition of CO_2 is the total concentration of carbon dioxide in the soil at any definite interval of time known. The possibility of toxic doses, therefore, is not eliminated. Thus, in PARKER'S experiments (**140**) the concentration of carbon dioxide was sufficient to increase markedly the absorption of silica. Evidence exists that different plants differ in sensitivity to CO_2 (**45, 179**), although in the culture solution experiments of HALL (**56**) wilting occurred when CO_2 was aspirated through all culture solutions. STOKLASA (**182**) and also LUNDEGÅRDH (**102, 103**) found that a concentration as low as 1 per cent. of CO_2 in the soil atmosphere 15 cm. below the soil surface retarded growth, and was even toxic to some plants.

It is of interest to note here that PFEIFFER and BLANCK (**145**) argue from the negative results obtained by CO_2 additions in their "oder-sand" cultures in the presence of rock phosphate that the data afford proof that other acids are exuded by roots, because, although many of the plants with which they experimented were able to utilize rock phosphate quite well, the artificial addition of CO_2 gave in all cases decreased yields.

3. Root excretions other than CO_2

a. The divergent views.—The tendency of the European workers (**1, 28, 55, 120, 183**) and also of some of the American investigators (**52, 67, 68**) is to emphasize the importance of carbon dioxide exudation, and to regard the rôle of other acid exudations, relatively, of little significance, and, indeed, in some cases (**52, 55, 183**) to question their existence. Others (**28, 120**) have claimed to have found substances other than acids in the root exudations.

b. Distinction between excretions from living and dead cells.—Although the fact that acids may be excreted from dead cells was early recognized by Czapek (**28**), it may be questioned if, in planning some experiments (**12**), sufficient attention has been paid to the necessity for making conditions such that the excretions of living and dead cells could be differentiated. That this point is of importance is shown by the results of Johnson (**75**), who found marked differences in the quantities of Ca^+ and Cl^- absorbed from a $CaCl_2$ solution, in the case of live and dead beet cells. The dead beet cells removed only 26.2 per cent. Cl from the roots whereas living beet cells removed 43.7 per cent.; but the proportion of Ca absorbed was nearly the same in both cases.

Osterhout (**133**) and also Rudolfs (**160, 161**) have also called attention to the effect of hydrolytic dissociation of neutral salts and to the effect of the differential rate of ionic absorption. These secondary effects, due to differences in the permeability of living and dead cells, will be discussed later in this paper.

c. Root sap acidity as a basis for chemical methods of determining nutrient requirements.—(1) *The direct method of approach.*—The assumption that acids were exuded from roots served as a basis for the attempts of Dyer (**38**) and his school to ascertain the fertilizer requirements of soils. Dyer argued that if acids are exuded, the total acidity of the sap should be an approximate measure of its solvent action. The sap acidity was obtained by averaging the results of determinations for total acidity of the water extracts of the roots from over a hundred different species of plants. This was found to be equal approximately to a 1 per cent. solution of citric acid. Lemmermann (**96**) also adopted a similar criterion and, as we shall see later, the greater assimilatory ability of the Leguminosae compared with the Gramineae was attributed by him to the greater total acidity of the root sap of the former.

Information on the nature of the root exudations was also sought by Czapek (**28**) and Kunze (**89**) by bringing intact seedling roots in contact with litmus paper and noting the change in color. It has been suggested by Haas (**54**) and others, however, that it is not possible to reach definite

conclusions from this type of experiment because different roots may differ in their capacity to absorb the blue dye of litmus. Differential absorption cannot account for all the facts, for KUNZE (**89**) showed by titration in parallel experiments that the acidity exhibited was far greater than that indicated by the turning point of litmus. And there is no evidence in KUNZE'S experiments that the acids arise from injured or dead cells. It is clear, too, that KUNZE recognized the limitations of litmus paper as an indicator, for he states that in some plants the amount of acid lies below the sensitivity of litmus.

CZAPEK (**28**) attributed the red color obtained by pressing seedlings against blue litmus paper to the exudation of KH_2PO_4, and emphasized the fact that the exudation of this salt could not be explained by any injury to the cells of the root hairs because the same result was obtained in culture experiments where there could be no question of injury; but it is to be noted that CZAPEK did not find the drops of liquid—always to be observed on the roots of seedlings developed in a humid atmosphere—to be acid. PRIANISHNIKOV (**153**) also found traces of PO_4 in the root secretions of *seedlings, but not from full grown normal plants.*

The whole subject of the secretion of acids other than CO_2 was subjected to a critical investigation by STOKLASA and ERNEST (**183**) with *Hordeum vulgare* and *Zea mays,* using a different technique. The seedlings were allowed to develop for 15 days in distilled water and then transferred to experimental cylinders, through which 20 liters of sterilized air free from CO_2 were passed. In one series of experiments a mixture of O_2 and N_2 gas was used. Under these conditions CO_2 and a trace of H_2 was found, but no PO_4 nor SO_4. In view of the fact that a large number of investigators (**8, 13, 79, 94, 101**) had reported the presence of lactic, acetic and formic acids in the root exudations of plants, STOKLASA and ERNEST carried out a similar series of experiments from which they conclude that these acids were only exuded when the root system suffers from lack of oxygen and is the result of partial intramolecular respiration. When the supply of oxygen was sufficient only carbon dioxide was found. These experiments were carried out at a uniform temperature (20–22° C.) and observations were made for 10–20 days. Air containing α-radium emanations increased respiration considerably.

STOKLASA and also HALL (**55**) have argued, from an ecological standpoint, that it is improbable that such important nutrients as K and P_2O_5 would be exuded from the root system; but this position is untenable, for many experiments (**15, 207**) show very clearly that plants may return to the soil at some stage of their growth, usually at maturity, some of these important nutrient elements.

(2) *The indirect method of approach.*—Some investigators (**117, 145, 181, 192**), on the other hand, have sought a solution of the problem of acid exudation of roots by determining the assimilatory power of different plants for difficultly soluble materials, like rock phosphate, and HALL (**55**) has sought a solution by taking out a balance sheet between the acid and bases contained within the completely developed plant.

(a) *Mitscherlich's view.*—The conclusions from the indirect methods devised by MITSCHERLICH (**119**) and that adopted by PFEIFFER and BLANCK (**145**) are contradictory. MITSCHERLICH (**119**) maintains that his "Wirkungsfaktor" values are a constant characteristic for each manure (fertilizer) on all crops and all soils. This "law" of MITSCHERLICH is expressed by the equation

$$\frac{dx}{dy} = k(A - y) \tag{7}$$

$$\text{or } \log_e (A - y) = \log_e A - c(x + b) \tag{8}$$

where: c = "Wirkungsfaktor" or action produced by the nutrient element applied,
A = maximum yield when x is not limiting,
x = amount of nutrient element added,
b = amount of available nutrient already present in the soil,
y = yield obtained by application of nutrient element x.

He argues, therefore, from the constancy of c that all plants must have at their disposal the same agent for the decomposition of difficultly soluble material and that this is CO_2 exclusively. In recent years he appears to have changed his views to some extent as a result of the following experiment (**120**), which is easily carried out. When a very dilute solution of HCl (0.0001 N) is added to root hairs observed under a microscope, at first local swelling up of the protoplasm can be observed, followed by a rupture and expulsion of the protoplasm into the surrounding medium. After some time *new root hairs are formed,* showing that the injurious effects of too high hydrogen-ion concentration have been overcome. These phenomena may, in part, be explained by the tendency of plants to change the reaction of the medium in the direction of neutrality as observed by PANTANELLI (**138**), HOAGLAND (**64, 65, 67**) and THERON (**185**). The medium might be neutralized by the absorption of hydrogen-ions and the excretion of OH ions simultaneously, or, as MITSCHERLICH believes, the injurious effect of the acid may be counter-balanced by the liberation of substances of the nature of organic buffers. These ideas would coincide with MAZÉ's (**109**) and also COMBER's view (**26**) that substances other than those of acid nature take part in the solubilization of mineral nutrients. That considerable organic matter is liberated from the root sap and root hairs sloughed off by plant roots is shown by the observations of LYON and WILSON (**105**), but

these findings are not applicable as evidence to the exudations of intact root hairs. MINIMA'S investigations (**116**) afford some evidence, although indirect, that organic acids are excreted by roots, on the basis that the period of maximum excretion of non-volatile organic acid buffers is coincident with that of maximum acidification of the soil.

MITSCHERLICH, in his earlier papers (**118**), held the view (**146, 147**) that the amount of P_2O_5 absorbed from rock phosphate by oats was identical with the amount dissolved by a saturated solution of carbon dioxide, and concluded (**117**) that the different "Aufschliessungs-vermögen" of different plants could be explained by differences exhibited in their ability to develop root systems, which, in turn, is proportional to the amount of carbon dioxide respired by them. More recently he has modified these views and has discarded chemical methods as being inadequate (**119**). Indeed, it has been found by numerous workers, especially by KÖNIG (**82**) and SCHLOESING (**168**), that oats and other plants may absorb far more P_2O_5 from rock phosphate and other soil phosphates than can be dissolved by a saturated solution of CO_2. FREAR and ERB (**43**) also drew similar conclusions with respect to the soil K_2O of the fertilizer plots of this Experiment Station.

In evaluating these opposing claims, it should be remembered that STOKLASA (**180**) and STOKLASA and ERNEST (**183**) found that lactic, acetic, formic and oxalic acids were excreted only when the root system suffers from lack of oxygen; but as yet there are no critical experiments indicating whether or not under average field conditions, the system in immediate contact with the roots may not be relatively deficient in oxygen. The accumulation of these organic acids due to incomplete oxidation of the products of respiration has been found by STOKLASA to be highly toxic to plants. This view is further supported by MAZÉ'S experiments (**109**) with peas in sterile cultures. That the problem of air renewal is a serious problem, especially in some of the finer textured soils, is apparent from technical remedies such as drainage and crop residues devised to meet it.

(b) *Hall and Miller's experiments.*—One of the arguments used by HALL (**55**) upon which to substantiate his conclusions that only CO_2 is excreted by the plant is based on the findings of HALL and MILLER (**57**) that the net action of the plant upon the soil is to leave an excess of base in the soil. This result was arrived at by taking a balance sheet between the acid and bases contained within the completely developed plants (wheat, barley, sweeds and hay) of the Rothamsted rotation experiments on the Agdell field, and also of wheat and cabbage plants grown in culture solutions. These experiments, however, do not take into consideration the effect of hydrogen-ion concentration. As THERON (**185**) and others have empha-

sized, greater equivalent proportions of anions than of cations are absorbed on the acid side and on the alkaline side the reverse holds true. If HALL'S statements had been made later, they undoubtedly would have been modified as a result of the recent progress in physical chemistry. Furthermore, the validity of the assumption made by HALL that all the nitrogen in the plant should be calculated as an acid on the basis that all the nitrogen enters the plant as nitrate may be questioned.

(c) *Kappen's experiments on expressed root saps.*—The procedure of KAPPEN (**77**) of determining the total acidity and hydrogen-ion concentration of the expressed sap of the roots of mature plants grown in field experimental plats is more pertinent; but even in these experiments values cannot be regarded as absolute, because—as we shall see later—of the existence of a hydrogen-ion concentration gradient (**53, 195**). Moreover, KAPPEN'S experiments are open to the criticism that they do not distinguish between diffusion products and exudations of the roots. Considered, however, from the standpoint of the relative comparisons between total acidity and hydrogen-ion concentration of the respective root saps, KAPPEN'S data indicating low hydrogen-ion concentration values but high titration numbers are instructive. Only 0.1 per cent. of the hydrogen-ions were found to be present in the ionic condition, the remainder being in the undissociated form. The inevitable conclusion is that the root saps must consist of a mixture of free organic acids and their alkali or alkali-earth salts. This point is of interest because such an arrangement would ensure the organism against too violent fluctuations in the degree of acidity of its sap, and is analagous to the conditions that exist with other physiological fluids, blood, urine, etc. Both GROH (**49**) and PAVLINOVA (**143**) come to the same conclusion as KAPPEN, but the deductions of the latter, unfortunately, are of limited application because only seedlings were used. They are of interest, however, in that they show that the older technique adopted by CZAPEK (**28**) is useless to determine the reaction of the water exuded by guttation.

(d) *Haas's experiments.*—HAAS (**52**) sought a solution of the problem of root exudation by determining simultaneously the hydrogen-ion concentration of solutions in which seedling plants were growing and also in controls without plants. After the CO_2 had been expelled by a current of pure H_2, the hydrogen-ion concentration of both series of cultures was identical. Although this method eliminates the possibility of confusing the exudation of living cells from those of dead cells, it is doubtful if the conclusion drawn by HAAS from these experiments, *viz.*, that plants do not exude other acids than CO_2, can be extended to *mature plants*. The mineral nutrient requirements of the 10 day seedlings with which HAAS worked are, of course, much less than in more mature plants.

C. The influence of the degree of the acidity of the cell sap

In view of the emphasis placed by many investigators upon the influence of the degree of the sap acidity of cells as a determining factor in the feeding of plants, it is pertinent for us to inquire more closely into this phenomenon.

1. Does a hydrogen-ion concentration gradient exist

Although there is sufficient evidence to indicate that the hydrogen-ion concentration of the substrate has an indirect influence on the growth of plants, it is not definitely known what, if any, relation exists between the hydrogen-ion concentration of the medium and that of the metabolically active tissues. Jacobs (**73, 74**), in the case of isolated cells, found none. Hoagland's results (**66**) on barley grown in water, in sand, and also in soil cultures indicate that the pH values of the sap of the tops were almost identical, even though the pH of the nutrient media varied widely, *viz.*, from 4.94 to 6.76 in water cultures and from 7.03 to 7.34 in soils. Reed and Haas (**157, 158**) report similar results from water culture experiments. The latter (**157**) and also Theron (**185**) have obtained comparable results in the case of the sap of tops, but in the roots Theron (**185**) noted that the pH increased with increase in pH of the medium (substrate). The existence of a definite hydrogen-ion concentration gradient in the case of algae has been shown by Child (**23**) and in some of the higher plants, grown in soils, by Haas (**53**) and in animal tissues by Gustafson (**51**). The regulatory influence of buffer systems, therefore, must vary considerably in different parts of the plant. The data of Haas (**53**) are illuminating. In *Melilotus alba* (second year growth), the following pH values were obtained: Soil extract, 7.68; root (6″ portion below upper 2″), 5.82; root (2″ of upper part), 6.46; leaves and petioles, 7.04; stems, 6.68. The pH of the mixed upper 3″ of the tops, stems, leaves and buds was 8.0. As Reed and Haas (**158**) later pointed out, any hypothesis based upon differences of acidity of the plant sap and the external medium must account not only for the accumulation of inorganic elements within the roots but also for their movements to other portions of the plant. Investigators in this field must, moreover, take cognizance of the fact that marked change in the hydrogen-ion concentration usually occurs within an hour after expression of the plant sap (**25, 50, 61, 195**).

From the foregoing, it is apparent that we are not yet in possession of sufficient evidence to determine whether or not the cell sap of tissues possessing similar functions have hydrogen-ion concentration values that may be characteristic of the species. The assumption that root sap acidity values are a characteristic of the species formed the basis of much of the experimental work by the German investigators, (**1, 89, 96**) in their search

for a solution of the causative factors determining the "Aufschliessungsvermögen" of different species. Thus, the Gramineae and the Leguminosae were supposed to be sharply differentiated (**96**) on the basis of the acidity of their root sap, until KAPPEN (**77**) and later STOKLASA (**181**) showed very definitely that the distinction held neither for the titration nor hydrogen-ion concentration values. STOKLASA's experiments (**181**), if confirmed, would lead to the conclusion that the acidity of the sap of the roots of all cultivated plants is near neutrality, except when insufficient oxygen is present, under which latter condition the acidity increases. He emphasizes the significance of this fact for the development of the bacteria of the rhizosphere.

As we shall presently see, working on this assumption, TRUOG (**194**) considers that the feeding power of different plant species is a function of the different intensities of the acidity of the respective saps. Thus, buckwheat and clover are cited as types of plants with high and low acidities, respectively. The table below, however, taken from the data of TRUOG and MEACHAM (**195**), KAPPEN (**77**), HAAS (**53**), STOKLASA (**181**), and ABERSON (**1**), will show how impossible is such a classification, at least with the methods hitherto adopted.

TABLE III

pH OF BUCKWHEAT AND RED CLOVER ROOTS REPORTED BY SEVERAL INVESTIGATORS

INVESTIGATOR	BUCKWHEAT	RED CLOVER
TRUOG and MEACHAM	4.0 – 4.03	5.6 – 5.9
KAPPEN	4.9 – 5.3	6.2
ABERSON	5.7 – 5.9	7.9 – 8.4
HAAS	4.8 (mature plant) 5.4 – 5.9 (seedlings)	5.8 – 6.1
STOKLASA	6.2	6.6

Further development of NĚMEC's work (**125**) on the hydrogen-ion concentration of the sap of seeds of a large number of different species of plants promises—from the practical standpoint—to clarify the whole situation. Seeds are more nearly constant in composition than other parts of plants, and NĚMEC found that the hydrogen-ion concentrations of the seeds of plants adapted to acid soils are relatively much higher than those adapted to alkaline soils. It is of interest to note that the saps from the seeds of barley, corn and peas were found to have the lowest hydrogen-ion concentration, pH 6.45 – 6.30.

It has been a matter of long observation that such plants as sweet clover, alfalfa and lettuce are adapted to grow on soils at or near the neutral point.

When soils to which these plants are adapted are allowed to go unlimed, they are replaced by others that are less sensitive to acid conditions. CAMARGO *et al.* (**19**) have recently shown in controlled experiments that the optimum growth of roots of the coffee plant is at pH 4.2, which affords an explanation of their adaptability to acid soils. The coffee plant is greatly injured by even moderate liming.

D. Theories proposed to explain differences in the feeding power of plants

1. THE APPLICATION OF THE LAW OF MASS ACTION

TSCHIRIKOW (**196, 197**) stressed the importance of the CaO/P_2O_5 ratio in the plant as an important factor in the ability of different plants to supply themselves with enough P_2O_5 from difficultly soluble phosphates. Plants having a high energy absorption for Ca would, according to him, depress the Ca concentration of the soil solution, resulting in an increased solubility of the phosphate. The smaller the amount of P_2O_5 necessary for the formation of one unit of organic substance of the plant (*i.e.*, the smaller the percentage of P_2O_5 in the plant), the greater obviously would be the effect of P_2O_5. From this viewpoint the capacity of the plant to absorb difficultly soluble phosphates is directly parallel to the quantity of Ca absorbed and inversely parallel to the P_2O_5 requirement of the plant.

TRUOG (**192, 194**) has extended TSCHIRIKOW's views further and has invoked the concept of equilibrium between calcium phosphate and carbonic acid, on the one hand, and the products of their interaction, on the other, as expressed by the equation:

$$Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows Ca_2H_2(PO_4)_2 + CaH_2(CO_3)_2 \qquad (9)$$

He contends that plants such as buckwheat, which the majority of culture experiments show is capable of utilizing the phosphorus of rock phosphate in large measure, are supposed to be able to absorb both of the products to the right of the equation. It is to be inferred, moreover, as already stated (p. 462), from TRUOG's later expansion of this view that the rate of absorption is determined by the degree of acidity of the saps of these plants. Thus, buckwheat is characterized as a plant with "high internal acidity." In the case of plants having a low degree of sap acidity, one of the products to the right of the equation (calcium bicarbonate) is assumed to be incapable of absorption by the plant at a rate comparable with that of the other product (di-calcium phosphate); consequently, the operation of the reverse reaction will prevent the further solution of the rock phosphate. Corn and oats are placed in this class by TRUOG.

This view has been supported (**6, 7, 53**) and as vigorously disputed (**30**). In order to clarify our ideas, it should at the outset be remembered

that, although the possibility (p. 456) of absorption of salts in the undissociated form (**133, 135, 137**) must be considered possible under some conditions, *e.g.*, in the case of detached cells, the weight of evidence (**67**) is that absorption in normal intact plants takes place in the ionic condition. Now there is ample evidence, as we shall see later, to show that not only do root hairs of different species differ in their degree of permeability to the same ion, but that the different relative velocities of the anion and cation of a salt may also be a factor in determining the equilibrium. We must then be prepared to consider ionic equilibrium, although the existence of such equilibria between the ions in the cells and the ions in solution has not as yet been established definitely.

2. Evidence for and against Truog's views

a. Bauer's sand culture experiments.—The results of Bauer (**6**) have been cited in support of Truog's views. In these experiments corn was grown in sand cultures treated with rock phosphate (or acid phosphate) as the source of P, and $NaNO_3$ or $(NH_4)_2NO_3$ as the source of N; but the analytical data are insufficient to show that the improvement in growth in the leached pots was due to removal of calcium as the bicarbonate. Removal of toxic substances by leaching must also be considered a factor (**119**). Further critical experiments on this point should include pots without plants as controls.

b. The views of the California group.—Davis, Hoagland, and Lipman (**30**) cite Newton's (**126**) results with peas and barley in water culture solutions, which grew normally without bicarbonates, and, moreover, in solutions at hydrogen-ion concentration at which bicarbonate ions could not exist to any extent. But this argument may be weakened by the fact that Hoagland (**67**) and others have later deduced evidence to show that rapid replacement of $-HCO_3$ ions by other ions may occur.

Reed and Haas (**157**) have also argued against Truog's view on the basis of their experimental work with walnut and orange trees. In experiments with the former the effect of a solution of $Ca(OH)_2$ of pH 9.0 was determined. Although the percentages of Ca in the ash of the tops and roots were 14.60 and 9.80, respectively, the pH of their sap (5.26–5.48) showed no significant changes from values obtained when such plants were grown in complete culture solutions. This same argument, however, used by Reed and Haas to refute the existence of a relation (**193**) between the absorption of calcium and the degree of acidity of the sap, breaks down completely when certain experimental data (**78**) may be interpreted, by the same method of reasoning, to prove that calcium may be necessary for precipitating organic acids formed as a by-product, on the basis that

mottled leaves of citrus trees starved of calcium are considerably more acid and contain distinctly less calcium than normal leaves.

Again, REED and HAAS (**158**) are inclined to the belief that there is no evidence in support of the assumption by TRUOG (**194**) that absorbed materials are converted into insoluble compounds as rapidly as they accumulate in the plant, or that absorption *necessarily depends* on the precipitation of ions within the plant. The italics are the writer's. But this conclusion is not justified from the experimental data supplied. Thus, REED and HAAS (**158**) find that in sand cultures the concentration of water-soluble K increases in the ash of leaves, trunk, shoots and stems, and that the concentration of soluble Ca increases in the leaves *but decreases* in the shoots, trunk and roots as the concentration of the nutrient solution increases. In soil cultures 45.82 per cent. of the Ca was soluble in the leaves, 15.03 per cent. in the shoots, and only 7–9.5 per cent. in the trunk, roots and rootlets. In buckwheat nearly all the calcium is insoluble in water (**67**). The fact that *Nitella* (**69**) contains large quantities of soluble potassium and calcium salts has also been used as an argument (**30**) against TRUOG's views. But to what extent the extension of data obtained from such abnormal structures as *Nitella* to the higher cultivated plants is justifiable remains to be determined.

The work of KOSTYCHEV and BERG (**88**) has an important bearing in this connection. They have determined the forms in which Ca is present in the tissues of a number of plants. The following interesting data, table IV, are taken from their paper:—

TABLE IV

FORMS OF CALCIUM IN PLANTS

PLANT	PERCENTAGE OF THE TOTAL CA		
	SOLUBLE IN WATER	COMBINED WITH PO_4 AND HCO_3	CA OXALATE
Trifolium repens (leaves)	42.4	38.6	19.0
Alchemilla sp. (leaves)	35.6	40.3	24.1
Caragana arborescens (leaves)	21.0	23.1	55.9
Solanum tuberosum (leaves)	16.5	34.5	49.0
Lemna minor	11.6	45.7	42.6

In apple tree experiments (**190**) the writer has found in mature leaves relatively low percentages of the total Ca soluble in water, *viz.*, 17.2 per cent., and relatively high percentages as the oxalate, *viz.*, 54.3 per cent. The above data might, therefore, be just as readily used to support TRUOG's views as to refute them.

CHIBNALL (**22**) has recently shown that one-tenth of the water-insoluble calcium of cabbage leaves consists of calcium phosphatide.

c. SOME WEAK POINTS IN TRUOG'S THEORY.—

(1) *The experiments of Parker—Bauer—Bartholomew.*—According to TRUOG (**194**) plants with a slightly acid or neutral sap, like sweet clover and alfalfa, are more able to utilize the potassium advantageously from lower concentrations of the element than plants, like buckwheat, having a more acid sap; but PARKER (**139**) found that buckwheat absorbed relatively large quantities of soil potassium. Moreover, BAUER (**6**) found that both buckwheat and sweet clover readily absorbed calcium from rock phosphate. BARTHOLOMEW (**4**) also failed to find any relation between the amount (percentage) of calcium absorbed and the ability of these plants to feed on rock phosphate, although the substrate and nutrient medium were the same as that used by TRUOG (quartz sand).

(2) *The effect of the destruction of the buffer systems.*—No account has been taken of the buffer systems present, the destruction of which must be of greater influence than the factors already noted. The buffer action of calcium bicarbonate, for example, is a function of the hydrogen-ion concentration of the medium, and is readily destroyed if the hydrogen-ion concentration varies from pH 7.5.

$$Ca(HCO_3)_2 + NaOH = CaCO_3 + NaHCO_3 + H_2O \qquad (10)$$

$$Ca(HCO_3)_2 + H_2SO_4 = CaSO_4 + 2H_2O + 2CO_2 \qquad (11)$$

The destruction of $Ca(HCO_3)_2$ results, as shown by BUTKEWITSCH (**16**), in increased yields—in some cases as much as three times—and the amount of calcium in the medium decreases. The stronger the buffer action the greater the CaO/P_2O_5 ratio. From this standpoint, the inhibitory action of $Ca(HCO_3)_2$ on phosphate utilization may be regarded as that of paralyzing the root activity of the plant. *In this sense and in this sense only may* TRUOG'S *view hold.*

(3) *Do the terms "high" and "low" acidity have any meaning?*—The validity of TRUOG'S conception must necessarily rest also upon the accuracy of his classification of the relative acidities of the saps of plants into high, medium and low; but, unfortunately, we have no definite knowledge of the relative acidities of plant saps. As we have seen, the variation in different parts of the same plant is so great that it is not, as yet, justifiable to use the terms "high" and "low" in classifying plant saps. The hydrogen-ion concentration of the sap of seeds is much more constant; therefore, in any attempt to correlate hydrogen-ion concentration of plant tissues with nutrient supply, the examination of the sap of seeds would be the most profitable line of attack.

d. LACK OF HARMONY OF OBSERVATION WITH THEORY.—Nevertheless, it is highly improbable that the results upon which PARKER and TRUOG (**142**) based their classification of plants into (1) high Ca and high N, and (2) low Ca and low N are accidental, as has been contended (**129**). On the contrary, the preliminary studies of STORCK and RIPPEL (**134**) support the existence of such a relationship between Ca and N. True, the analytical data presented by PARKER and TRUOG represent the composition of plants grown under very varied and different conditions. But an examination of the paper (**142**) will show at once that the data have been collected with great care and, moreover, TRUOG has himself conducted carefully controlled experiments. He recognizes (**193**) that isolated analyses may be found that are relatively quite different from those used—especially of plants that have reached maturity or were cut as hay—due to losses of Ca, in part possibly by leaching (**95**).

If we accept this relationship between calcium and nitrogen as an actual fact, to what may it be attributed? Surely not to acids arising from the decomposition of proteins, as TRUOG assumes. The older physiologists (**33**) considered that "living" protein substance of the protoplasm functions as the immediate material for respiration, resulting in the production of the acids found in plants. But, as pointed out by KOSTYCHEV (**87**), this assumption contradicts an abundance of facts that have been established recently without question—that sugars are much more easily oxidized than proteins. Most of the plant acids arise from the carbohydrates and fats that serve as the fundamental sources of energy of respiration (**156**). As a matter of fact the amount of energy obtained by the decomposition of proteins is very small, and, moreover, the amino acids formed are readily converted by the plant again into proteins (**176**). Only after the complete consumption of sugars do plants begin to burn the protein material of their protoplasmic framework, and then the CO_2/O_2 ratio falls to 0.70–0.80.

The explanation of the Ca/N relations observed by PARKER and TRUOG (**142**) is more probably to be explained by the antagonistic action of Ca and K resulting in an increased absorption of N and P_2O_5 (**188**).

e. EXTENT TO WHICH BASES FUNCTION AS NEUTRALIZERS OF PLANT ACIDS.—TRUOG and MEACHAM (**195**) and also HAAS (**53**) have examined the hydrogen-ion concentrations of the tops of a large number of plants grown both in limed and unlimed plots, and, without considering the possibility that the results are within the limits of experimental error (**185**), it was found that with few exceptions the pH of the tops of the *limed* plants was higher than that of the *unlimed*. It might be argued from these facts (**53**) that one function of calcium is to adjust the pH of the soil solution so as to

enable the plant to secure a sufficiently rapid supply of bases to neutralize the injurious effect of too high acidity; but, apart from the fact that neither CLEVENGER (**25**) nor DUSTMAN (**37**) have been able to corroborate such a conclusion, there is absolutely no justification to assume that this function is more peculiar to calcium than the other bases, *e.g.*, magnesium and potassium (**90, 91**). Moreover, it is not necessary to assume that the disappearance of acids is accomplished solely by the mechanism of neutralization, since ASTRUC (**2**) has shown conclusively that the disappearance of acids—which are always higher in the young plants—is due not so much to their "neutralization" as to the process of respiration and esterification.

f. CALCIUM REQUIREMENTS OF PLANTS ARE VERY DIFFERENT.—It is certain that plants cannot continue growth without calcium and, although different species respond differently to its presence, injury does not result if sufficient quantity of soluble salts are present in the media (**157**). The observation of LOEW (**114**), therefore, of the death of cells on treatment with salts that precipitate Ca must be interpreted accordingly.

The response of species to varying quantities of calcium appears to be an important ecological factor (**201**). Thus, SKEEN (**175**) has shown that *Phaseolus vulgaris nanus* must have large amounts of calcium and that *Lupinus albus* obtains its maximum growth with relative traces.

From the foregoing, we are forced to conclude that, although the suggested explanation (p. 467) offered to account for this Ca/N relationship may not explain all the facts, there is no sound reason for denying the existence of this relationship. It is unfortunate, from this standpoint, that the nitrogen content of the oat plants grown by SHEDD (**171**) on different soils and which showed such extreme ranges in calcium content are not known. This would have given us some valuable information.

3. CONCLUSIONS FROM RECENT MORE DIRECT EXPERIMENTS

RUSSELL (**162**) considers that the critical problem is to know whether the concentration of the substrate (nutrient solution) with respect to calcium has decreased to a greater extent in the "high feeding" plants than in the "low feeders." Such experiments are at present being carried out by DOMONTOVITSCH and SCHESTAKOW (**34**), of which only the preliminary results have been published to date. In sand culture experiments with lupines, buckwheat, mustard, millet and oats—using rock phosphate (size of particles 0.1 mm.) as the source of P—the growth of the plants and concentration with respect to P_2O_5 and Ca were compared between pots containing mixed plants, *viz.*, (1) buckwheat and oats, (2) lupines and oats, (3) lupines and millet, (4) mustard and oats, and pots containing an equal number of plants grown alone. The following table V gives the results of the preliminary work:—

TABLE V

DATA FROM DOMONTOVITSCH AND SCHESTAKOW

NR. DER GEFÄSSE	P_2O_5—QUELLE UND PFLANZENART	pH	P_2O_5 IN 100 CC. DER LÖSUNG MG.	CA IN 100 CC. DER LÖSUNG MG.
4	Rohphosphat Hafer	7.43	0.020	95.0
16	" Hirse	7.14	0.018	111.93
6	" Buchweizen mit Hafer	5.43	0.041	.....
5	" Buchweizen	6.25	0.036	.....
11	" Lupine	5.34	0.767	30.85
12	" Lupine mit Hafer	5.17	0.679	75.89
17	" Lupine mit Hafer	4.80	1.350	48.59
26	" Hafer	7.21	Spuren	.....
36	" Senf mit Hafer	6.35	grössere Spuren	.....

The growth of oats and millet was scanty and not much greater than those plants without any phosphorus addition. In the mixed cultures with lupines the yield of oats and millet was increased 545 and 846 per cent. respectively, over the yields of these plants grown alone. Mustard and buckwheat also influenced the growth of these Gramineae to a remarkable extent. Unfortunately, calcium determinations are not reported in all cases; but it is evident that the mixed cultures with lupines have reduced the concentration of the calcium under that in the pots in which the Gramineae grew alone. The hydrogen-ion concentration of the medium in the mixed cultures is also much lower than that in the cultures of plants grown alone. These results indicate that the differences in the utilization of rock phosphate in these experiments are to be correlated with their effect on the hydrogen-ion concentration of the root medium and, perhaps, also with the unequal rates of absorption of calcium. The data for concentration of P_2O_5 show no relation between the rates of absorption of phosphate ion by the different plants.

HOAGLAND has recently reported that with the same source of rock phosphate, buckwheat was able to make normal growth, whereas tomato plants were almost entirely unable to obtain phosphate from this source—yet the tomato plants had a much higher percentage of calcium than the buckwheat plants (**68**). The conclusion is drawn that the solid phase of the soil and its ability to keep the soil solution supplied with calcium is an important factor. Results obtained by MAZÉ (**109**) may be similarly interpreted. It would be of interest for investigators in this country to conduct experiments similar to those of DOMONTOVITSCH and SCHESTAKOW's work and, in

addition, to determine the content of the plants in Ca and PO_4. It is well to bear in mind, however, the possibility that the carbon dioxide exuded by roots may, under the artificial conditions of many of the experiments reported, be of far greater importance than under normal conditions and for this reason it is undoubtedly more advantageous to use soils as the substrate.

E. The influence of the extent of the root system

1. Is there any relationship between feeding power and extent of roots

That great differences exist with respect to the extent of the root system among different types of plants is a common observation. A review of the literature has been given by Miller (**115**). This difference in the growth of plant roots outwards, together with the differences in their respiration energy, is regarded by some authorities (**30**) as an important factor in the mechanism of absorption of ions by plants.

Truog has also clearly recognized that differences in the extent of root system may be a factor in the absorbing power of plants. This is quite evident from a statement in one of his earlier papers (**193**) to the effect that "the feeding power of a plant for lime depends largely on the extent and character of the root system." From the results of experiments carried out later, Truog modified this view and, as we have seen, stressed the internal acidity of the sap as a more important factor in plants like buckwheat, which have a very restricted root system.

But there are several experiments (**84, 152, 154, 155, 192**) that indicate no evidence of the existence of any relationship between feeding power and the extent of the root system. In quartz sand cultures with rock phosphate as the source of phosphorus (**84, 152, 154, 155, 192**) much larger yields and a much higher percentage of P_2O_5 were obtained in the case of the legumes (peas and lupines) and also of buckwheat than of the cereals. In other words, in these experiments buckwheat—with a small root system—(**145, 146**) utilizes rock phosphate better than the cereals which have, relatively, a very extensive root system. Parker's data (**139**) are of interest in this connection. Comparing the absorption of sorghum and cotton under similar conditions, it was found that the latter absorbed more than double the amount of ash constituents than the former. If, as the majority of field observations tend to indicate, sorghum has a more extensive root system than the tap-rooted cotton plants, differences in absorption of mineral constituents cannot, in Parker's experiments, be attributed to differences in extent of root systems. On the other hand, Davis *et al.* (**30**) cite Newton's experiments (**126**) to show that the extent of root system is an important factor in the absorption of ions from soil. This conclusion is based on

NEWTON'S experiments in which peas and barley were grown together. Under these conditions the peas absorbed more calcium than barley from *soil cultures*; but there was no similar difference in *solution cultures*. It is argued, therefore, that if barley and peas differed considerably in extent of root system such results would be expected to follow. Data on the growth of the respective root systems in these experiments are not given. In a later experiment (**128**) on the evolution of CO_2 from barley and peas, the dry weights of the roots of peas were found to be less than those of barley. NEWTON himself (**128**), however, is inclined to attribute these differences to the much larger respiration energy of peas over barley than to differences in the extent of root systems. But HOAGLAND (**67**) later pointed out that comparisons of extent of root systems have only a very limited value, because the general appearance and size of a root system is not necessarily an accurate index of the total active absorbing area. In this connection it is of interest to note that RUSSELL (**162**) is of the opinion that the whole question of the supply of nutrients involves both root spread and replacement of ions, although no experimental evidence is cited.

Consequently, views on the relation of extent of root system to feeding power are necessarily speculative until more critical data are forthcoming. The method of determining the actual absorbing surface such as that employed by DUSTMAN (**37**) and others is unquestionably the most accurate. A critical discussion of the relative merits of the various procedures has been given recently by WEAVER and HIMMEL (**202**).

F. The influence of other factors

1. PERMEABILITY

In an earlier paper (**187**) the writer has discussed some of the factors involved in "membrane" permeability. Until sufficient quantitative and qualitative data, obtained under rigid control of all the variables are available it is improbable that views on the subject can be anything but speculative. Possibly, a satisfactory working hypothesis may be formulated only when data, based upon cataphoresis experiments, have accumulated from a wide variety of materials carrying no charge (**99**). The importance of securing more qualitative information is indicated by the interesting properties of the phosphatide ($RO_2 \cdot C \cdot CH_2OH(C \cdot R'O_2)CH_2O \cdot PO_3H_2$) isolated by CHANNON and CHIBNALL (**22**). It forms a fat-soluble calcium salt but a water-soluble sodium salt; and, accordingly, cell permeability could be conceived to be altered by a change in the proportion of calcium to sodium salts.

From thermodynamic considerations and also from the kinetic theory it can be deduced that ionic pairs having the higher mobility will pass through a membrane in proportionally the greater quantity, and also that

the permeability of an ion is increased by the introduction of another electrolyte possessing a common ion. Thus, many investigators (**100**) have found that the absorption of K from KCl is increased by the addition of NaCl; and the effect of the NaCl has been shown to be dependent on the hydrogen-ion concentration of the medium. Since hydrogen and hydroxyl ions have the greatest velocity, the addition of acids (decrease of pH) will increase the diffusion of anions; the addition of alkalies (increase of pH) that of cations (**46, 47, 48**).

The influence of the hydrogen-ion concentration of the medium on the absorption of *weak acids and bases and their salts* by living cells has been investigated by JACOBS (**73, 74**). The pH of the medium to which a cell is exposed was found to bear no necessary relation to the pH produced by that medium within the cell itself. The higher permeability of living membranes to undissociated acids and bases results in a higher physiological activity of weak acids and bases compared with stronger ones. Conditions favoring the formation of undissociated molecules, therefore, will promote their penetration into membranes to which they are permeable. The result of these influences may, as LOEB (**100**) has shown, be to modify the properties of the cell wall in such a way as to accelerate the rate of diffusion of certain ions and retard the rate of diffusion of other ions. $CaCl_2$ and NaCl are important factors in causing such selective diffusion.

From the practical standpoint it is interesting to note that STOKLASA (**183**) finds that different plants have different selective abilities towards cations and anions, respectively. The Gramineae, in his experiments, absorb anions more than cations; potatoes and beets more cations than anions; legumes anions and cations equally. Of course, further confirmation of such results is necessary.

2. ION ANTAGONISM

There is sufficient evidence to show that Ca and Mg, which precipitate sols, cause contraction and impermeability and that K and Na, which stabilize sols, cause relaxation on the one hand, and greater permeability on the other. The antagonistic effects between univalent and divalent cations in colloidal systems have been studied by MEULEN and RIEMAN (**113, 159**), by WEISER (**203**), and also by HÖBER (**71**). SIMMS (**173**) has derived a mathematical expression for the antagonism between Na (or K) and Mg (or Ca) in true (non-colloidal) solutions of oxalate. One might expect—although it has not been demonstrated—that the activity of proteins would show a similar antagonism.

It is essential, however, for the investigators to keep in mind the fact that the effect of a mixture of two or more cations (or anions) on cell permeability may be quite different from the effect of one alone when the activity of the ions is due to different combining proportions of the cations

(or anions) in a mixture (such as the soil solution), in proportion to the relative combinations in solutions of each alone (**174**). Some of the practical agricultural applications of ion antagonism between Ca and K have been investigated by LIPMAN *et al.* (**98**), by MCCOOL and WELDON (**106**) and by FONDER (**42**).

3. GIBBS-DONNAN DISTRIBUTION LAW

In systems containing a non-diffusible ion, a marked effect is observed on the unequal distribution of inorganic electrolytes on either side of the cell membrane. A non-diffusible electro-negative ion will produce a greater concentration of diffusible electro-positive ions and a lower concentration of electro-negative ions on its own side of the membrane than on the other. A non-diffusible electro-positive ion will produce the opposite effect. At equilibrium the product of the concentrations of each pair of oppositely charged diffusible ions is the same on either side (**35, 36**). The distribution of an electrolyte in such a system will be expressed by the equation:

$$\frac{x_i\, y_i}{v_i^2} = \frac{x_e\, y_e}{v_e^2} \qquad (12)$$

where x_i and y_i represent the amounts of cation and anion, respectively, within the membrane at equilibrium; and x_e and y_e the equilibrium amounts of the same pairs of ions in the external solution; v_i and v_e the volume of the solvent on either side of the membrane.

The importance that the GIBBS-DONNAN distribution law may have in the equilibrium conditions and consequent selectivity of nutrients by plants has been mentioned by HOAGLAND (**67**), PIERRE and PARKER (**148**), and also by BRIGGS and PETRIE (**14**). It will be recalled (**187**) that PARKER (**141**) found that the displaced soil solution from many of the experimental fields in America were too low in inorganic phosphorus to support growth in the nutrient media when the phosphorus in these soil solutions was used as the source of phosphorus. One of the explanations offered by him to explain this anomaly is that a DONNAN membrane equilibrium may exist, resulting in a higher concentration of phosphorus in the solution on the surface of the soil particles than in the soil solution itself. The mechanism involved has already been discussed by the writer (**132, 187**). But the exact experimental verification of DONNAN's law in the case of specific plant tissue is difficult, for it is impracticable to measure the x_i, y_i, and v_i accurately, since a calculation of the amount of ions absorbed by means of the difference between their concentration before and after equilibrium is reached does not take cognizance of the amounts of these ions already present in the tissue (**14**). Hence, extension of the methods of OSTERHOUT (**135**) and of OSTERHOUT and DORCAS (**137**) on the absorption of CO_2 by *Valonia* do not afford a basis for generalization, for even here the average

concentration of the ions in the cell, as a whole, is not considered but only that in the cell sap.

As BRIGGS and PETRIE (**14**) point out, in the plant cell we are dealing with a polyphase system. The DONNAN equilibrium applies to free ions only. In the cell there is evidence that some of the ions are present as undissociated salts of proteins or other substances, or again some of the ions may be physically absorbed. We should expect, therefore, that the simple DONNAN equation would not hold in such systems. That this is the case is apparent from the results of BRIGGS and PETRIE's experiments on the absorption of 0.1 M KCl and NH_4Cl from carrot tissues (**14**) and from WRIGHT'S (**214**) work on the secretion of high amounts of calcium in milk from the relatively low concentration of this element in the blood plasma. The latter finds that both the degree of dissociation of the protein salts and the establishment of a DONNAN equilibrium must be taken into account in explaining the inequalities of the products in the distribution of inorganic elements in living tissues, whereas the former (**14**) have deduced that the apparent ionic internal product, resulting from the collective effect of all the phases within the tissue, must have a higher value than that of the external media. BRIGGS and PETRIE (**14**) point out that "the plant cell is not a simple system, composed of a mere membrane enclosing a homogeneous solution, one of the ions of which is indiffusible; there are at least three phases between which the ions may be distributed—the external medium, the cytoplasm, and the vacuoles. Within the cytoplasm also, and perhaps in the cell-sap, there are micelles, or gel-particles, which can constitute yet other phases."

BUTKEWITSCH and BUTKEWITSCH (**17, 18**) have advanced as a reason for the inequality of the products of the diffusible ions on each side of a membrane in systems containing a non-diffusible ion, that the degree of permeability of the plasma membrane is not the same in both directions. This has been shown to be the case by WERTHEIMER (**204, 205**) for certain types of membranes. In such a case the equation of membrane equilibrium will contain, besides the concentration product of diffusible ions, a factor corresponding to the velocity of migration of the corresponding ionic pairs. Thus, if a solution, say of KCl, is separated by a membrane permeable in the two directions, we have:—

$$\begin{array}{cccc} x' & K^+ & K^+ & x'' \\ y' & Cl^- & Cl^- & y'' \\ & p' \rightarrow & & \\ & \leftarrow p'' & & \end{array}$$

where $x'\ y'\ p' = x''\ y''\ p''$

where x and y represent the concentration of K and Cl ions and p a permeability factor.

By this means it can be calculated (**17**) that in OSTERHOUT'S experiments on *Valonia* (**134**) the permeability of the plasma membrane for Na in one direction is one-fifth that in the opposite direction and in the case of K the difference is 45 times.

Within the limitations already discussed it is possible to interpret the experiments of BUTKEWITSCH and BUTKEWITSCH (**17**) on the influence of colloidal silicic acid upon the absorption of phosphorus by maize plants by DONNAN'S principle. When small amounts of P_2O_5 are present in the nutrient medium, growth and absolute amounts of P_2O_5 increased by the additions of silicic acid in the nutrient medium; but the *percentage* of P_2O_5 remained about the same. It follows that a functional replacement of P_2O_5 by H_2SiO_3 in the plant does not occur, but that the favorable action of H_2SiO_3 on the development of the plants is due to its effect in increasing the absorption of P_2O_5 in a medium that contained minimum amounts of phosphorus for growth (**189**).

4. THE EFFECT OF DIFFERENCES IN POTENTIAL BETWEEN SOIL AND PLANT

Since the breaking down of carbohydrates sets free electromotive force, part of the energy stored in the plant must be electrical and used in growth. The work of GROH (**49**) is important. He attributes the different behavior of different plants toward different degrees of hydrogen-ion concentration in the medium to a difference in potential between the plant and soil solutions, which results in a decomposition of the nutrient material. This hypothesis is supported by the following experiment. Peas and also barley were grown in sand and also in soil cultures to which $Ca(NO_3)_2$ were added. During growth individual plants were connected with a galvanometer with one of the platinum electrodes immersed in the soil and the other connected to the plant. The E.M.F. produced varied with conditions—from 0.01 to 0.20 volts. The greater the distance from the roots, of the electrode connected to the plant, the greater the current registered. This shows that the difference of potential existing in the plant itself is much less than that existing between the plant and the soil. The degree of vegetative development apparently was not a large factor in determining the degree of the difference of potentials noted. GROH'S work, although of a preliminary nature, is suggestive and should be considered in connection with OSTERHOUT'S (**136**) more refined work on the potential difference in *Valonia* and *Nitella*.

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ELECTRIC CORRELATION POTENTIALS IN THE LEAF OF *BRYOPHYLLUM*

E. J. Lund and M. Bush

(with three plates and nine figures)

Introduction

It has long been known that some forms of living tissues have the peculiar property of producing a continuous output of electrical energy under normal conditions of growth and development. These continuously existing differences of potential have been studied more extensively in plants than in animals by early investigators. Buff (**3**), Kunkel (**7**), Elfving (**5**), Burdon-Sanderson (**4**), and Müller-Hettlingen (**14**). Later Mathews (**13**) found that differences of potential occurred in hydroid stems of *Tubularia, Pennaria,* and *Campanularia,* and that the structural polarity of these hydroids was accompanied by electrical polarity. More recently, the magnitude, direction, and distribution of maintained electric potentials have been determined in the stem of *Obelia,* Lund (**8**), and in onion roots, Lund and Kenyon (**11**). Later experiments showed a quantitative relation between cell oxidation and continuously maintained bio-electric currents in such polar tissues as *Obelia,* frog skin, and roots, Lund (**9**). These findings have led to the formulation of a theory which expresses the relation between organic polarity, cell oxidation, and continuously maintained differences of electric potential within the same cell or organism. In the onion root, Marsh (**12**) and in the Douglas fir and white fir, Lund (**10**), it has been demonstrated that each cell in any given length of tissue is the seat of an individual E. M. F. such that the potential difference which occurs between two points on the tissue, represents an algebraic sum of all the individual E. M. F.'s of the cells in the region over which the potential is measured.

The following experiments were carried out for the purpose of extending the observations on continuously maintained differences of electric potential to a diversity of living material, and to further test the principle of summation of E. M. F.'s.

Plants, such as *Coleus, Cosmos,* and *Bryophyllum,* were used in preliminary experiments, and all of them exhibited continuously maintained differences of potential along the main axis. *Bryophyllum* was selected for further experiments. This well-known plant furnishes especially interesting material because of its peculiar habit of producing shoots from growing points at the notches of the leaf after it is detached (plate V).

In the experiments which follow, different pairs of points on the leaf were selected for measurement of potential difference in order to determine:

(1) whether continuous electric potentials were maintained between the growing points, (2) whether each growing point developed a potential independently of other growing points, and (3) whether the growing points differed from other parts of the leaf in the development of inherent electric potential. The final problem was to determine whether or not evidence of algebraic summation of E. M. F.'s would be found in suitable preparations of the leaf.

Experimental material consisted of potted plants of *Bryophyllum* in various stages of active, normal growth.

Methods and apparatus

Potentials were measured by a Leeds and Northrup hydrogen ion potentiometer. Readings were expressed in millivolts. The limit of accuracy under the conditions of the experiments was less than one millivolt.

The arrangement of the apparatus is shown in plate VI. A moist chamber, A, was supported on a stand equipped with rack and pinion, F. In the center of the floor of the chamber was a paraffined cork on which the leaf preparation, L, rested during experiments. In the floor of the chamber were five perforations to admit the tubes from the electrode cups.

The glass tubes from the electrode cups, D and E, were joined by rubber tubing to bent glass tubes, B and C, which ended at the tip in two claw-like glass projections. The claw fitted loosely over the growing point and insured a good water contact. If one contact was to be made at a surface of a leaf preparation, then a straight tip, with a capillary opening was used instead of the bent claw. The electrode cups were supported by means of special adjustable clamps which were attached to stands, G and H, each equipped with rack and pinion. The clamps and the rack and pinions facilitated the adjustment of the contact tips. The electrode cups and tubes were filled with tap water which formed contacts with the leaf preparation. The water in the cups was kept level with that at the contact tips.

Positive and negative electrodes, P and N, respectively, dipped into the cups as represented. The electrodes were of cadmium amalgam-$CdSO_4$ type.

The leads from the electrodes passed to K which is a large paraffin block supported by a wooden block, and which contained six cups filled with mercury. The cups were connected by copper keys with sealing wax handles.

The potential difference of the electrode system was measured each time and subtracted from the potential difference found when the leaf preparation was in the circuit. It was necessary to take the measurement of the electrodes rather frequently, since the potential difference of the electrode system changed somewhat when an experiment was carried on for several

hours. The variation was 0.5 to 1.5 millivolts in two or three hours when the $CdSO_4$ electrodes were used.

After a leaf preparation was placed within the moist chamber, and the electrode tips were adjusted, a few minutes were allowed to elapse in order to permit the leaf preparation to recover from the stimulation which resulted from the cutting and the placing on the electrode contacts. Care was then taken not to touch the preparation during an experiment. Since the moist chamber, A, was lined with moist filter paper, the amount of water at the contacts remained constant. Any renewal or changing of the water at the contact "stimulated" (?) the tissue at the point of contact. This was verified by making measurements of the potential difference before renewing a contact and immediately afterward. The latter showed a much greater variation and more rapid changes in potential than those before the contact was renewed.

Experimental

In preliminary experiments, measurements of difference of potential were made on *Coleus, Cosmos,* and *Bryophyllum,* particularly between different points along the length of the stems. In order to measure the potential from the entire stem, a little strand of wet cotton was placed around the stem at the level of measurement and contact was made with the cotton. The cotton also prevented the possibility of the electrode tip coming in contact with the stem and injuring or stimulating it. The older, basal, hardened parts of the stems of *Cosmos* and *Bryophyllum* show smaller differences of potential than corresponding lengths of the newer, actively growing parts of the same stem. These observations are in agreement with the observations on roots, the *Obelia* stem, and the Douglas fir.

The special experiments which follow, were made with *Bryophyllum* leaves, or parts of leaves.

1. Differences of potential between the petiole and selected points on the surface of the leaf blade

Experiments were performed using the entire leaf and measuring the differences of potential between the distal part of the petiole and (1) each of four selected growing points (plate VII, fig. 1), and (2) each of four selected points on the ventral side of the leaf (plate VII, fig. 2). In each case these measurements were made while the leaf was attached to the plant, and again when the leaf was detached. The petiole was wrapped loosely with a little strand of wet cotton, and rested between the glass projections of the electrode tip. The results of these and following experiments are recorded by means of graphs. In these, the abscissa represents time in minutes and the ordinate represents readings in millivolts. In figures 1, 2, 3, and 4 the

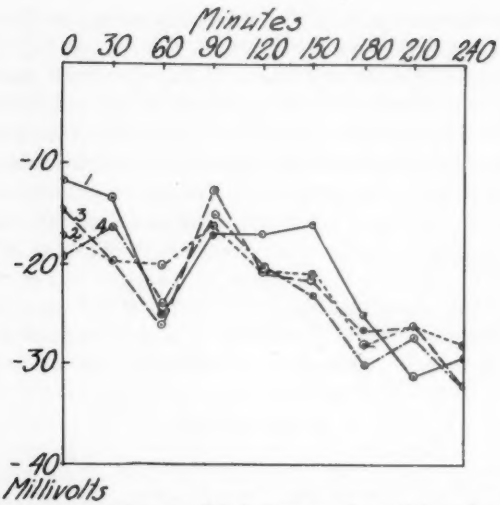

FIG. 1. Curves representing the P.D.'s between the petiole and each one of four selected growing points while the leaf was attached to the plant. Curves 1, 2, 3, 4 correspond to the P.D.'s between the points 1, 2, 3, 4, and the point on the petiole marked + in plate VII, 1.

negative signs indicate that the growing points and the selected points on the lamina are negative to the distal part of the petiole. The numbers of the graphs correspond to the numbers of the points indicated in plate VII, figures 1 and 2.

Figure 1 represents the differences of potential between the distal petiole and each of four selected growing points, while the leaf was attached. Figure 2 (a) represents the differences of potential between the same points immediately after the leaf was detached. Figure 2 (b) represents the differences of potential between the same points five days after the leaf was detached, when the growing points were beginning to sprout.

The leaf removed showed greater variations than the same leaf on the plant. It showed somewhat greater differences of potential a short time after being detached than it did five days later. The temperature of the room was constant to within two degrees during these tests.

Figure 3 represents the differences of potential between the distal part of the petiole and four selected points on the ventral side of the leaf, while the leaf was attached. Note that the potential differences are less variable than those between the growing points and the petiole. Figure 4 represents differences of potentials between the same points after the leaf was detached. In this experiment also, the detached leaf showed an increase in

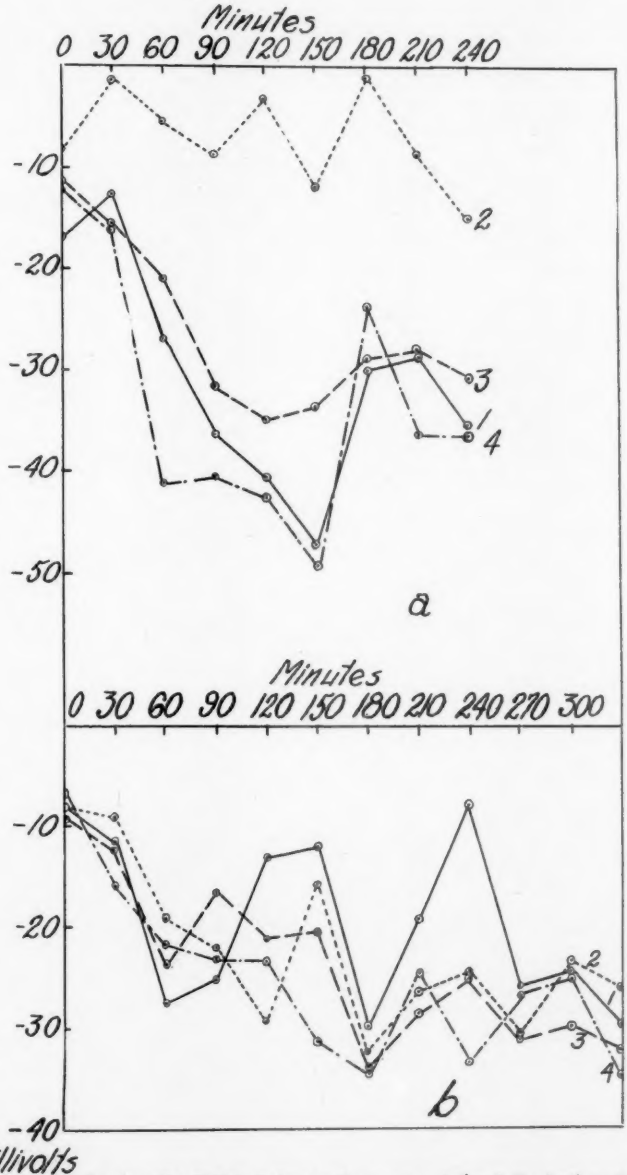

FIG. 2. *a*. Curves showing the P.D.'s between the same points of the leaf as those in fig. 1, immediately after the leaf was detached. *b*. Same as *a*, but taken five days after the leaf was detached. Note the difference between the different points.

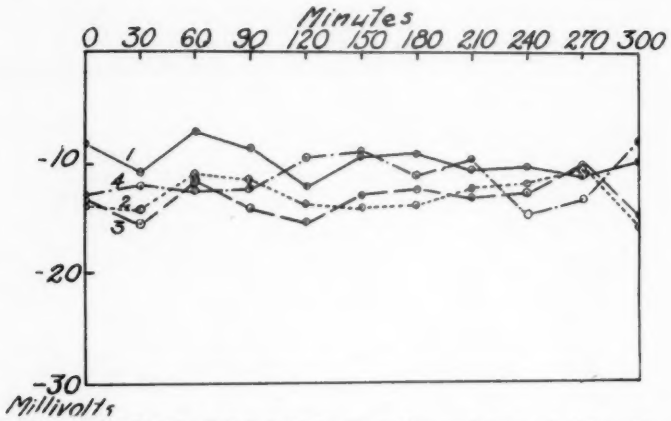

FIG. 3. Curves showing the P.D.'s between the petiole and four selected points on the ventral side of the same leaf as that of figs. 1 and 2, while the leaf was attached to the plant. Compare with curves of fig. 1.

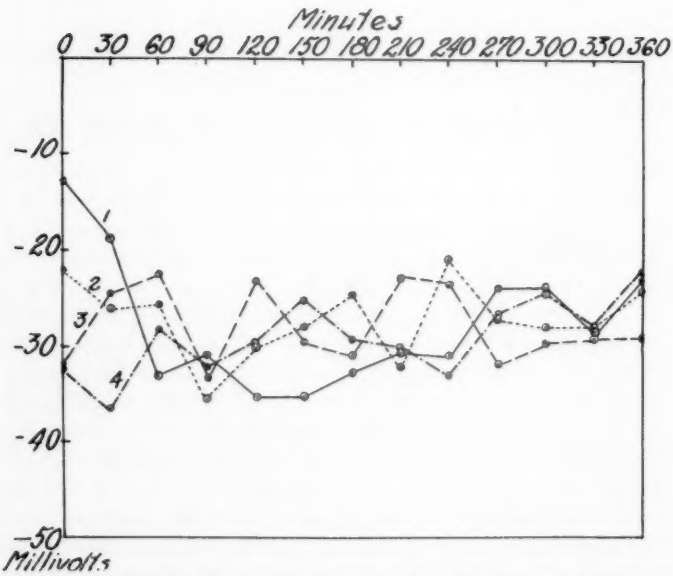

FIG. 4. Same as fig. 3, after removal of the leaf from the plant.

differences of potential and in variation, the room temperature being the same during the tests.

These experiments show the petiole to be positive to the growing points and to points on the lamina. They also show that under what may be considered constant external conditions (absence of external "stimulus") the processes which develop the maintained electric potentials, change from instant to instant. In this respect they resemble the potentials in roots. The curves show that the potential differences between different growing points and the same point on the petiole are within certain limits independent variables. The curves clearly indicate that in general local processes determine the magnitude of local potentials, or, in other words, the observed potentials are the algebraic sum of a complex system of E. M. F.'s of diverse, internal, local origins. The results amplify and confirm the observations on roots, *Obelia,* and the Douglas fir.

In previous work on electrical phenomena in plants and animal tissues it has been the practice to ignore electric potentials of the type referred to in this paper, if they were not overlooked. Often if such potentials were observed, an attempt was made to compensate for them by applying a counter electromotive force before the application of an external stimulus, the effects of which were the objects of the study. A typical example of such procedure is found in the work by FRASER (**6**). Here emphasis is therefore placed upon the fact that the causes of the observed variations are largely if not entirely within the tissue system itself.

2. DIFFERENCES OF POTENTIAL BETWEEN TWO GROWING POINTS ON OPPOSITE SIDES OF A LEAF AND BETWEEN TWO ADJACENT GROWING POINTS

A narrow strip of a leaf, containing two growing points from opposite sides of a leaf was prepared (plate VII, fig. 3). Figure 5, a and b, represents the result of the readings from two such preparations. As shown by the graphs, the points were alternately positive and negative in relation to each other. The magnitude of potential at a growing point varied continuously so that, sometimes, one of the growing points had a greater potential, and was, therefore, positive to the second. Then, again, the second growing point showed a higher potential and became positive to the first. Zero indicated that the potentials of the two growing point systems were equal and oppositely directed and, consequently, there was no deflection of the galvanometer.

A similar preparation was made, but instead of having the growing points connected by leaf tissue, they were connected by a water bridge (plate VII, fig. 4). The bridge consisted of a narrow piece of filter paper which prevented the water from spreading. In this preparation also, the growing

points alternated positive and negative as shown in figure 6. This experiment showed that the growing points were not dependent on each other in the development of potential, since they were not connected by leaf tissue.

A preparation consisting of a narrow strip of leaf with two adjacent growing points was made (plate VII, fig. 5) and potential differences between the two growing points were measured. The path of the circuit in this case was in the leaf tissue between the two growing points. Figure 7 represents the results of a typical experiment. The potential of the growing points in this preparation also alternated positive and negative in relation to each other.

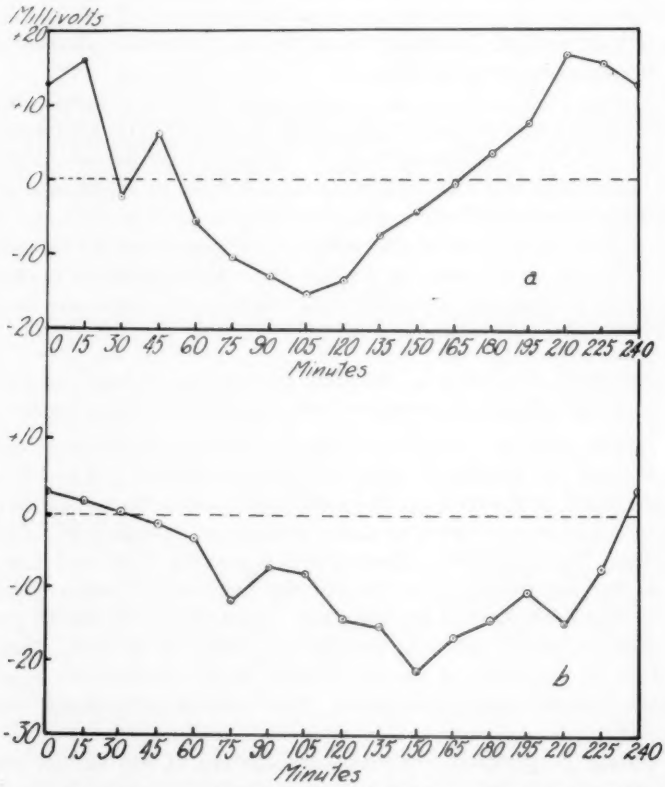

FIG. 5. *a.* and *b.* Curves showing the P.D.'s between oppositely directed growing points of two different leaf preparations as shown in plate VII, 3. Note the spontaneous reversals of orientation of total E. M. F.

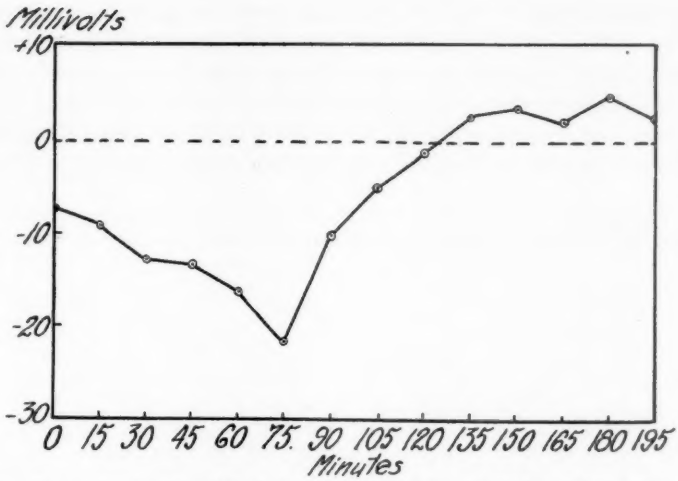

FIG. 6. Curve showing the P.D. between two opposite oriented growing points when the tissue pieces were connected as shown in plate VII, 4.

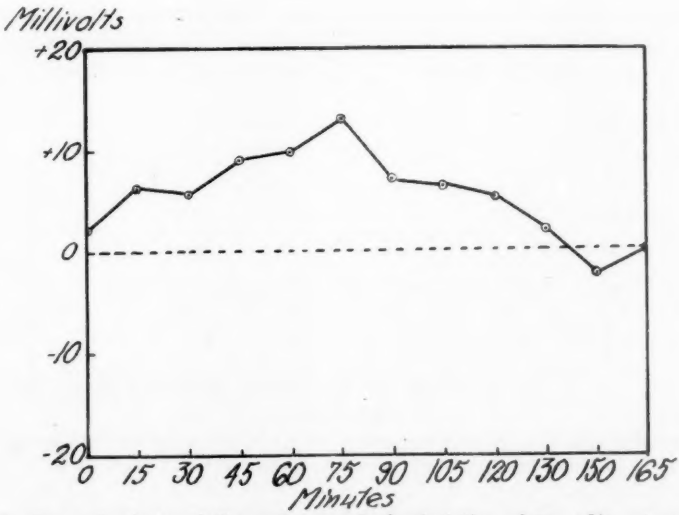

FIG. 7. Curve showing variation and reversal of orientation of two adjacent growing points as shown in plate VII, 5.

3. Differences of electric potential between growing points and other points on the leaf surface

In order to determine the relation of potentials of the growing points to the other parts of the leaf, measurements were made, (1) between a growing point and a point on the lamina (plate VII, fig. 6) and (2) between a growing point and the tip of the adjacent lobe (plate VII, fig. 7). Figure 8

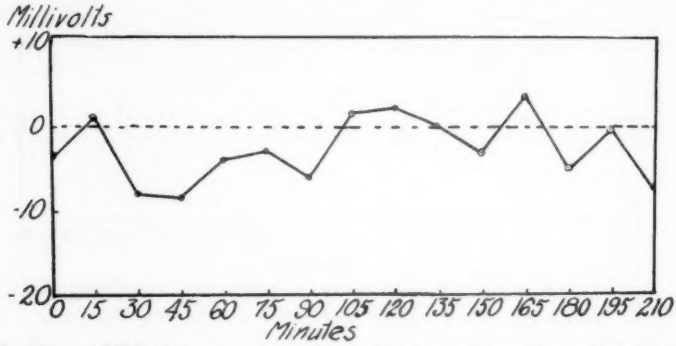

Fig. 8. Curve of P.D. between a growing point and a point on the ventral side of the leaf as shown in plate VII, 6. Note that the growing point is not always positive.

shows the results of one series of readings. The growing point was not always positive to the point on the lamina. In the second series of measurements, represented by figure 9, the growing point was without exception

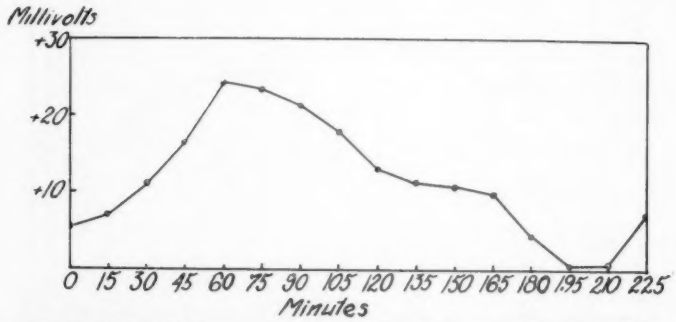

Fig. 9. Curve of P.D. between a growing point and the tip of the lobe between two adjacent growing points as shown in plate VII, 7. See table I.

positive to the tip of the adjacent lobe. Many measurements were made using this sort of preparation, four or five readings at fifteen minute inter-

vals were made on each one of these preparations, but no reading showed the growing point negative to the tip of the lobe. Results of these readings are given in table I. These results show without a single exception that the growing point develops an electric potential greater than, and independent of that produced by the surrounding tissue.

TABLE I

DIFFERENCES OF POTENTIAL IN MILLIVOLTS BETWEEN A GROWING POINT AND A POINT AT THE TIP OF AN ADJACENT LOBE. SUCCESSIVE MEASUREMENTS ON THE SAME LEAF STRIP TAKEN AT FIFTEEN MINUTE INTERVALS

PREPARATION	1*	2	3	4	5	6
	+ 1.8	+ 2.5	+ 3.3	+ 3.0	+ 5.3	+ 3.5
	+ 3.6	+ 4.8	+ 9.3	+ 6.5	+ 7.3	+ 8.2
	+11.2	+ 9.5	+11.0	+ 8.2	+10.6	+ 8.0
	+12.8	+10.2	+12.3	+10.2	+19.0	+ 6.5
Average	+ 6.8	+ 6.7	+ 8.9	+ 6.9	+10.5	+ 6.5
PREPARATION	7	8	9	10	11	12
	+ 3.0	+ 2.1	+ 9.5	+ 9.4	+ 7.0	+ 3.4
	+ 7.2	+ 8.1	+ 1.5	+ 4.8	+ 7.2	+ 8.4
	+ 6.2	+ 7.6	+ 5.5	+ 5.6	+ 8.5	+ 9.6
	+ 9.5	+ 9.4	+ 6.0	+ 4.8	+10.5	+16.1
Average	+ 6.4	+ 6.8	+ 5.6	+ 6.1	+ 8.3	+ 9.3
PREPARATION	13	14	15	16	17	18
	+ 4.0	+ 4.2	+ 3.0	+ 5.0	+ .8	+ 2.5
	+ 5.4	+ 7.8	+ 7.5	+ 8.5	+ 3.5	+ 4.5
	+ 8.3	+12.0	+15.0	+16.8	+ 6.5	+ 8.0
	+14.5	+20.0	+19.5	+20.0	+16.0	+19.0
Average	+ 8.0	+11.0	+11.2	+12.5	+ 6.7	+ 8.5
PREPARATION	19	20	21	22		
	+ 1.0	+ 3.0	+ 2.3	+ 5.5		
	+ 2.5	+ 5.0	+ 5.7	+ 7.7		
	+ 8.8	+12.5	+ 9.5	+ 9.5		
	+10.0	+15.7	+17.5	+18.0		
Average	+ 5.5	+ 9.0	+ 8.7	+10.1		

* The numbers at the tops of the columns indicate preparations (leaf strips).
+ The + sign indicates that the growing point is positive to the point on the lobe.

4. Evidence for algebraic summation of E. M. F.'s in a strip across a leaf of *Bryophyllum*

In the first experiment, a preparation consisting of two growing points on opposite sides of the leaf, connected by a very narrow strip of leaf tissue was used (plate VII, fig. 8). The electrode tips with glass projections were fitted loosely at A and C, and B rested between the glass projections. By trying out various combinations of positive and negative electrodes, it was found that A and C were each positive to B, and in this particular experiment, A was positive to C in all readings made. The measurements of difference of potential between A and B, B and C, and A and C are given in table II. The readings for A–B and B–C were subtracted algebraically and

TABLE II

Potential differences in millivolts between two growing points on opposite sides of a leaf, connected by a narrow strip of leaf tissue. (Contacts as shown in plate III, figure 8)

PD A——B + −	PD B——C − +	Expected PD A——C + −	Observed PD A——C + −	Difference in millivolts
+32.7	−48.0	−15.3	−10.5	4.8
+30.7	−50.3	−19.6	−15.8	3.8
+27.0	−43.7	−16.7	−14.0	2.7
+32.3	−41.1	− 8.8	−10.0	1.2
+30.3	−37.1	− 6.8	−10.0	3.2
+29.5	−42.7	−13.2	−18.0	4.8

the result was compared with the actual reading for A–C. According to our limit of error, the difference between the expected differences of potential and the observed difference of potential should not have been greater than two millivolts. Since the results showed an average difference of 3.1 millivolts we may conclude that in this type of preparation, an exact summation did not occur. The differences were probably due to the fact that the electrode at B covered a relatively wide area, and to the fact that the negative potential from the cut edges of the preparation was in part entering into the measurements. The central part of the strip was now replaced by a short water bridge (plate VII, fig. 9). A straight electrode tip with a small opening supported the water bridge at B. This arrangement insured a constant potential at B. Measurements were made as before. The growing

points A and C were both positive to B and in the A–C measurements, C was positive to A. The readings taken are given in table III. At no time was the difference between the expected difference of potential and the observed difference of potential greater than the limit of error. The results of this experiment, therefore, illustrate in a simple way the possible condition of summation of electromotive forces in cells of the leaf.

TABLE III

POTENTIAL DIFFERENCES IN MILLIVOLTS BETWEEN TWO GROWING POINTS ON OPPOSITE SIDES OF THE LEAF, CONNECTED BY A WATER BRIDGE. (CONTACTS AS SHOWN IN PLATE III, FIGURE 9)

A——B + −	B——C − +	EXPECTED PD A——C − +	OBSERVED PD A——C − +	DIFFERENCE IN MILLIVOLTS
+42.6	−22.5	+20.1	+19.5	0.6
+43.6	−21.5	+22.1	+22.2	0.1
+43.2	−24.6	+18.6	+20.2	1.6
+47	−24.5	+23.5	+22.6	0.9
+42.8	−21.6	+21.2	+20.5	0.7
+50.2	−29.5	+20.7	+21.8	1.1
+47.8	−22	+25.8	+25.2	0.6

Discussion

The purpose of the foregoing experiments has been to present some of the general facts regarding the orientation, magnitude and variation of the inherent electric potentials in a leaf which possesses a system of growing points. The results show that the growing points on the leaf compose a dynamic system of individual electric polarities, whose variations in magnitude appear on the whole to be more or less independent of one another.

The observed electromotive force is without doubt an algebraic sum of a diversity of individual potentials of definite local origins. The potentials in the *Bryophyllum* leaf are fundamentally like those which occur in other polar growing structures, where the principle of summation of E. M. F. definitely applies, MARSH (**12**), LUND (**10**).

A study of the curves showing the simultaneous variations of potentials in different parts of the leaf and growing points forces the conclusion that in the leaf of *Bryophyllum* there exists a complex but characteristic system

of continuous electric currents of variable magnitude. The electrical energy of these currents is continuously dissipated and supplied from specific types of biochemical processes, probably cell oxidation (basal oxidation?) LUND (9).

If the leaf of *Bryophyllum* were immersed in an electrically conducting medium, then in a limited manner the approximate distribution of such a system of currents could be visualized. The simplest types of such a natural condition are found in hydroids and roots immersed in their natural medium In aerial structures the pathways of the "return circuits" are of course not indicated by such experiments as those in the present paper nor those in previous papers on the Douglas fir.

It has been suggested that the currents produced in the leaf may be photoelectric in origin. Many plants do produce photoelectric currents, in the production of which the activity of chloroplasts under the influence of light, plays a prominent part WALLER (15). These currents doubtless are related to plant metabolism in the process of carbohydrate manufacture by photosynthesis. The bioelectric currents in the experiments described in this paper are not photoelectric currents since they are produced in the dark as well as in light and since the points of highest potential are not the points of greatest photosynthetic activity in the leaf.

In a study of the structure of the growing points of a leaf of *Bryophyllum,* BEALS (1) found that the root and shoot arise from division of small phloem cells of the vein near the vegetative point in the leaf. This fact is particularly interesting since BOSE (2) has suggested that the phloem is the electromotively active tissue of a plant under stimulation. Perhaps the phloem is a specialized tissue which produces, in addition to the meristematic tissues the continuous bioelectric currents associated with growth processes.

Summary

1. The experiments which have been described show that a complex system of measurable, inherent, and continuously maintained electric potentials exist in the leaf of *Bryophyllum.*

2. In the experiments the distal part of the petiole was found to be positive to growing points and to selected points on the lamina.

3. Not all growing points are positive to any points on the lamina of the leaf. A point on the lamina close to a growing point may be positive to another growing point.

4. A growing point in the unstimulated condition was always positive to a point at the tip of the adjacent lobe.

5. The experiments indicate definitely that the total E. M. F. in a transverse strip isolated from the leaf and having a growing point at each end of

the strip is equal to the algebraic sum of a complex system of E. M. F.'s of individual cells of the strip. The results on the leaf of *Bryophyllum* extend and confirm the observations on roots, *Obelia,* and the Douglas fir.

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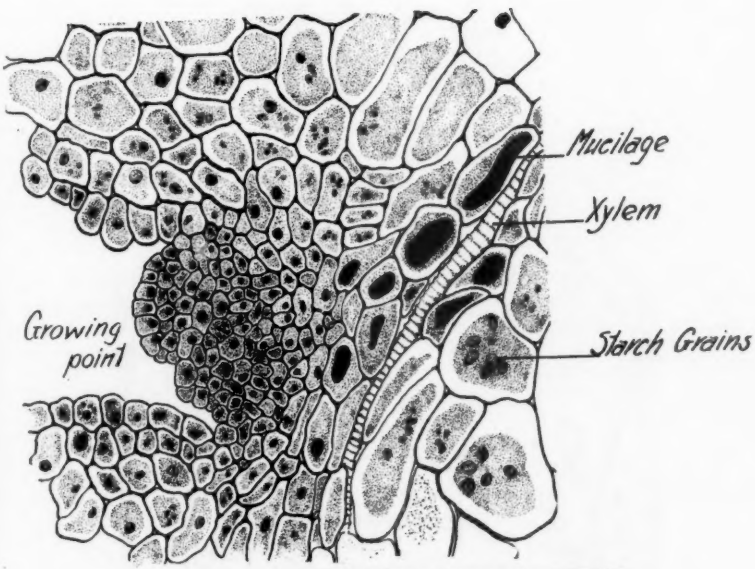
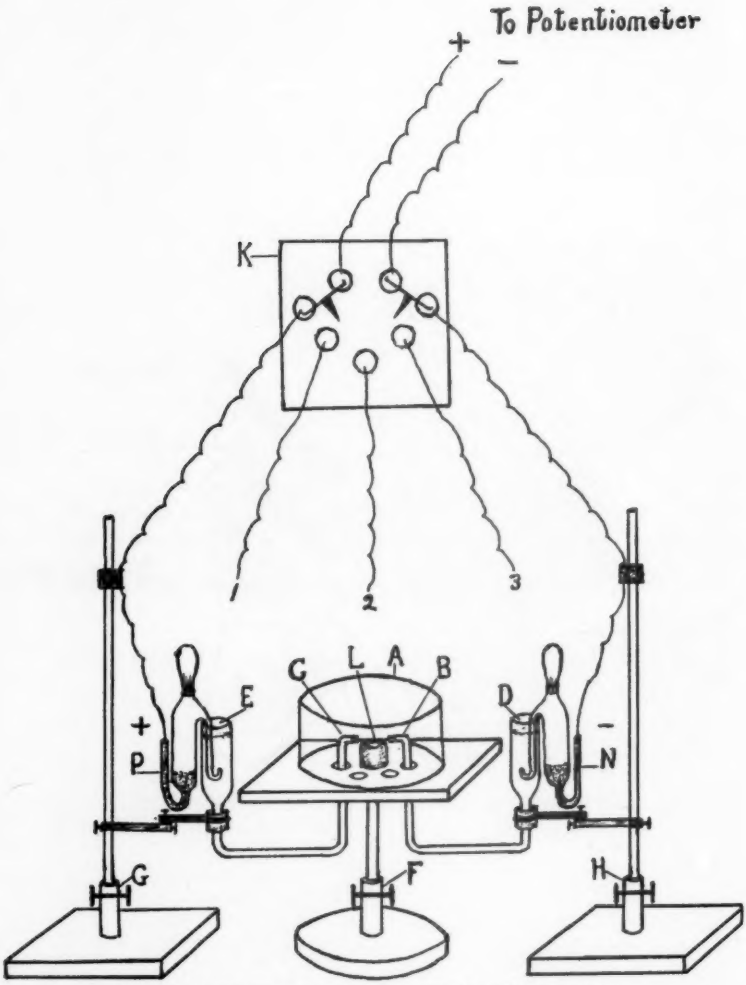
PLATE V

Plate V shows a horizontal section of a growing point from a leaf of *Bryophyllum* × 310. The growing point is located in the angle of a notch of the leaf and is embedded in leaf tissue. No branch of the vascular bundle reaches the growing point directly but branches are given off on each side of the notch. A few cells in the meristem tissue show mitosis. Many of the cells in the surrounding tissue show deposits of starch.



LUND AND BUSH—CORRELATION POTENTIALS



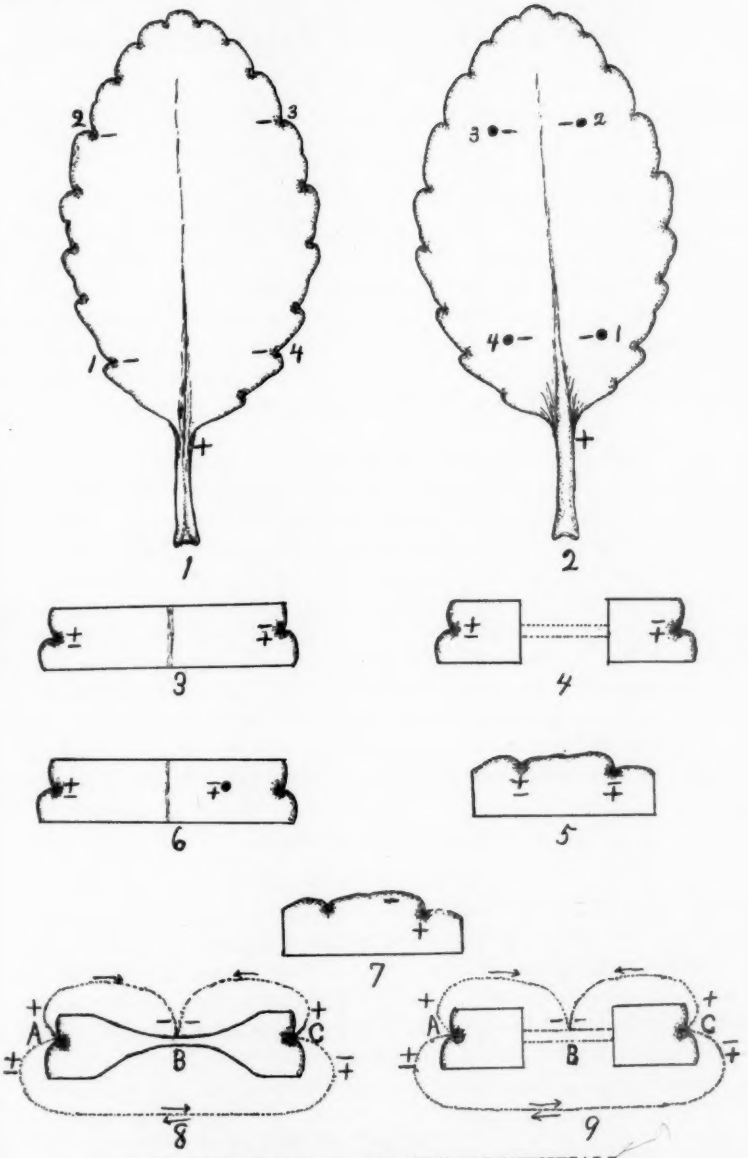
LUND AND BUSH—CORRELATION POTENTIALS

PLATE VI

Plate VI shows the arrangement of the apparatus with the wires leading to the key and to the potentiometer. Wires 1, 2, and 3 show possible electrode connections. These were used in the first five experiments when five electrodes were required to make the desired measurements.

PLATE VII

Plate VII shows the various leaf preparations and the position of the electrodes. In figs. 8 and 9 the directions of the currents are shown. The positive and negative signs refer to the outside circuit.



LUND AND BUSH—CORRELATION POTENTIALS

RESPIRATION OF APPLE TWIGS IN RELATION TO WINTER HARDINESS[1]

W. A. De Long,[2] J. H. Beaumont,[3] and J. J. Willaman[4]

(WITH SEVEN FIGURES)

The cold resistance of woody plants has hitherto been studied mainly from the static viewpoint. The problem has been attacked, in most cases, either by means of chemical analysis of whole ground tissues of hardy and tender plants or by microscopic analysis of the tissue structure. A minor amount of study has been directed towards microchemical determination of the distribution of food reserves within the tissues and the estimation of the seasonal changes in such food reserves. Some attention has also been devoted to measuring the amount of colloidal constituents and their degree of dispersion.

Life phenomena are, however, dynamic in character, and one of the most fundamental properties of living matter is its reaction to stimuli, or irritability. The fact of irritability may be demonstrated by the application of the most diverse agents, as anaesthetics, electrical shock, and changes in temperature. One of the most delicate means of showing that stimulation is so produced is by measurement of the changes which occur in respiration as evidenced by the rate of consumption of oxygen or of evolution of carbon dioxide. The present writers conceived the idea that respiration might throw some light on the phenomenon of winter hardiness in apple twigs. The present paper is a report of their findings.

It seems best to proceed directly with the description of the experiments, and to mention pertinent literature in connection with the discussion of our results.

Methods and apparatus

CO_2 production

The low temperatures desired were obtained by means of an ice-cream storage cabinet of the type described previously by us (**3**). Because of certain difficulties in temperature control encountered in the application of the air-bath method previously described, an oil bath was substituted in the second year of the work on the present problem. To this end the individual chambers of the cabinet were supplied with galvanized cans about one and

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one-half inches smaller in diameter than the chambers themselves and of such a height as to permit of the insertion into the top of the chamber of a cover carrying the necessary controls. These cans were filled with petroleum distillate. A brass pump of the type ordinarily used in water thermostats was placed in each chamber to maintain circulation. Heat was supplied as necessary by 110 volt, 125 watt electric heaters of the knife-blade type. The heaters were activated by means of the Harvey thermo-regulator relay system previously described (**3**, fig. 4). The temperature of the oil baths was thereby maintained constant within a range of about 0.1° C.

In all of the work presented here continuous aspiration was used, so as to avoid the effects of accumulated CO_2 previously emphasized (**14**). The carbon dioxide evolved was collected in absorption cells of the coil type already described (**3**, fig. 6), and absorbed in approximately 0.2 N standard NaOH. The semi-closed gas circuit system of circulation activated by an electric blower, previously described (**3**, p. 494) was used in the preliminary work of the season of 1926–7. Later, a one-way flow with compressed air was used.

In the case of the latter method the ingoing air was washed by passing through two consecutive absorption towers filled with 40 per cent. NaOH solution. These towers were fitted with absorption coils similar to those used in the measurement of the carbon dioxide evolved, with the exception that the central tube was lengthened at the lower end to the extent of about six inches in order that more pressure might be exerted upon the coil without forcing the air out at the bottom rather than up through the coil in the normal manner. This increase in length necessitated the use of larger containers for the coils, that is, about 1000 cc. The rate of flow was about 18 liters per hour through the washing tower, or 6 liters per hour for each of the three sets of absorption coils. The volume of the respiratory cells used being approximately 650 cc. when empty, or 450–550 cc. when filled with twigs, the rate of flow used assured a complete renewal of the gases in the system once every 10–15 minutes. For a carbon dioxide production of 100 mg. per kilogram-hour, which was about the maximum found in the present studies, the concentration of CO_2 in the atmosphere surrounding the twigs would not be in excess of 0.2 per cent.

After passing the washing towers, the air was led through a calcium chloride drying tower to prevent the freezing out of moisture at the low temperature points in the system. It was then passed over the samples of twigs and conducted thence to the absorption coils, two of which were placed in series in order to ensure complete removal of the evolved carbon dioxide from the outgoing gases. So far as possible all connections between the washing towers and the absorption cells were of copper tubing, the necessary rubber tubing joints being made as short as conveniently possible. The effi-

ciency of the washing towers, and the tightness of the joints were tested at frequent intervals during the course of the work. This was done by running the empty apparatus exactly as when in use for periods of 20 hours or more and determining the amount of carbon dioxide in the absorption cells at the end of that time. The average of all such blank runs was 0.04 mg. carbon dioxide per hour, which is well within the experimental error of the titration procedure for intervals of 10 hours or less.

The twigs were placed in copper respiratory cells similar to those of glass previously described by us (**3,** fig. 2) with the exception that the inlet tube was not coiled but simply passed down one side of the cell, across the bottom, and up the other to near the top, where it entered. The temperature within these cells was checked with a thermograph and found to be the same as that of the external bath, thus showing that the ingoing air was cooled to the desired extent during its passage through the straight inlet tube. As in the case of the glass containers, the outgoing gases were drawn off near the bottom of the cell. The top was closed with a heavy rubber stopper.

The samples usually consisted of 30 one-year twigs. These were placed in the respiratory cells as soon as possible after removal from the tree, the interval from tree to cell being about 30 minutes on the average and rarely more than 35 minutes. The cells were then immersed in the oil bath of the desired temperature, connected to the washing towers and absorption cells, and aspiration commenced immediately. Before titration, the carbonate in the absorption cells was precipitated with 10 per cent. $BaCl_2$ solution and the residual NaOH immediately titrated with approximately 0.1 N standard solution of HCl, using phenolphthalein. The results are uniformly expressed on the basis of the number of mg. of CO_2 produced per kilogram (fresh weight) of twigs per hour.

Chemical analysis of twigs

The twigs were ground in a motor-driven pencil sharpener immediately after removal from the designated storage chamber. The material, as soon as ground, was thoroughly mixed by shaking, and 25-gram samples withdrawn for analysis. These samples were weighed as quickly as possible on a balance accurate to 0.05 gram and immediately dropped into sufficient boiling 95 per cent. alcohol containing 0.2 gram calcium carbonate to make a final concentration of approximately 80 per cent. Heating was continued for one-half to three-quarters of an hour. The ground wood was subsequently extracted with alcohol in a Landsiedl extractor until a negative Molisch test was obtained. After making to volume the alcohol was removed from an aliquot by distillation under reduced pressure at a temperature of 40–50° C. After clearing with neutral lead acetate and removing the excess

lead with potassium oxalate, total and reducing sugars were determined on the aqueous filtrate. The QUISUMBING-THOMAS reduction method (**11**) was used and the reduced copper was estimated by permanganate titration after addition of saturated acid ferric sulphate solution according to the official methods of the Association of Official Agricultural Chemists. Acid-hydrolysable "starch" was determined on the alcohol-insoluble residue using the official method, except that reduction was carried out as indicated above.

RESPIRATION OF EXCISED TWIGS

In all cases in this paper the term "respiration" should be considered as synonymous with "CO_2 output," or "CO_2 evolution." On account of the very great experimental difficulties encountered by WILLAMAN and BEAUMONT (**13**) in their preliminary study of the respiration of apple twigs at low temperatures, in which measurement of the CO_2 production of twigs while still attached to the tree was attempted, it was decided to use only excised twigs in the present investigation. Such being the case it became of importance to determine to what extent the wounding of the twigs in their removal from the tree affected the subsequent CO_2 production. To this end uniform samples of twigs were collected at different times, divided into two portions, one of which was aspirated as collected while the twigs of the second portion were first cut in two, thus giving three cut surfaces per twig in place of one. Assuming that the effect of the second cut was practically identical with that of the first, one-half the difference between the amounts of CO_2 evolved by the first and second portions of the sample would be equal to the amount liberated from the cut surfaces and produced as the result of wound stimulation. This proved to be a relatively small and fairly constant fraction of the total, namely, 5–8 per cent. as shown by the data presented in table I.

EFFECT OF CHANGE OF TEMPERATURE ON THE RESPIRATION RATE.—When excised twigs are held at a constant temperature the rate of CO_2 evolution becomes fairly constant after 20 or 30 hours, but after 150 or 200 hours a steady decline is noticed. In this paper, however, we will deal with time periods of less than 30 hours, and in this case we do not have an equilibrium rate.

Of the first two samples of twigs investigated, the one (A) was held at $+6°$ C. and the other (B) at $-2°$ C., and it was found that while the respiratory level of (B) became relatively constant after the first few hours of aspiration, that of (A) continued to show a very appreciable falling off in rate even after 200 hours. It was then decided to reverse the temperatures of the two samples in order to determine the effect of small temperature changes upon the intensity of respiration at this low level. The result

TABLE I

EFFECT ON CARBON DIOXIDE OUTPUT OF CUTTING TWIGS

VARIETY	No. OF CUTS	DATE	TOTAL CO_2	CO_2 PER KG.	DIFFERENCE	TOTAL CO_2, DUE TO ONE CUT
			mg.	*mg.*	*per cent.*	*per cent.*
Duchess	One	Feb. 29	194	916		
	Two		197	1019	+11.2	5.6
Florence	One	March 4	195	1269		
	Two		250	1446	+14.0	7.0
Hibernal	One	March 14	134	789		
	Two		152	919	+16.5	8.25
Hibernal	One	March 24	409	2601		
	Two		449	2948	+13.4	6.7
Hibernal*	One	March 31	58	378		
	Two		66	416	+10.2	5.1
				Mean difference (per cent.).....		6.53 ± 0.26

* Aspiration at −10° C. All the preceding determinations were made at +6° C.

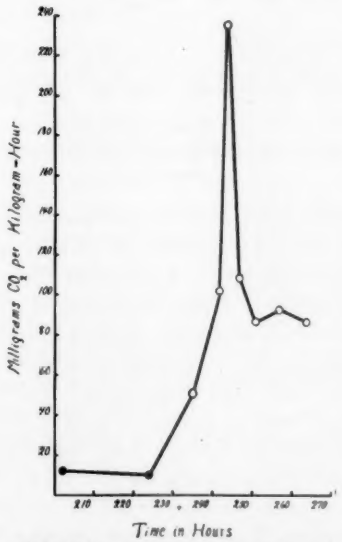

FIG. 1. Output of CO_2 by apple twigs at 6° C. after storage at −2° C. Solid dots represent measurements at −2° C., and circles those at 6° C.

of changing sample (B) from $-2°$ C. to $+6°$ C. is represented graphically in fig. 1, a conclusive demonstration that small upward variations in temperature within this range were capable of greatly accelerating this respiratory rate. On the other hand, changing the temperature of sample (A) from $+6°$ C. to $-2°$ C. merely caused the rate of respiration to fall to a level practically identical with that previously exhibited by sample (B) at that temperature.

These results were repeatedly confirmed. Fig. 2 contains representative curves of respiration over periods of 90 hours, when the temperature of

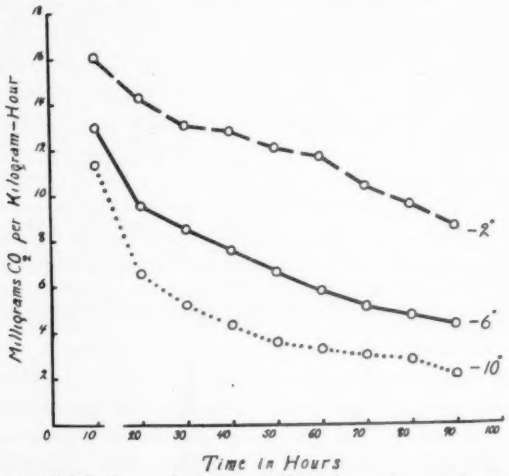

FIG. 2. Output of CO_2 by apple twigs at three lower temperatures after storage above 0° C.

the respiration period is *lower* than that of the previous period. It will be seen that there is an immediate falling off in CO_2 output, with a tendency to assume a level after some time. On the contrary, when the temperature of the experimental period is *higher* than that of the previous period, the CO_2 production always rises to a peak within a few hours, and then declines to a more or less constant value. Furthermore, the lower the previous temperature, the greater is the peak production. This is illustrated in fig. 3.

If this phenomenon obtains generally, it is obvious that, in attempting to determine the respiratory rate of plant material, cognizance must be taken of its previous temperature history. We thought that one way of determining whether this is the case would be to take samples at intervals during the winter, noting the temperatures during the previous 24 hours, and measuring the rate of CO_2 production at 6° C., which temperature would be higher than that of the orchard for all samples.

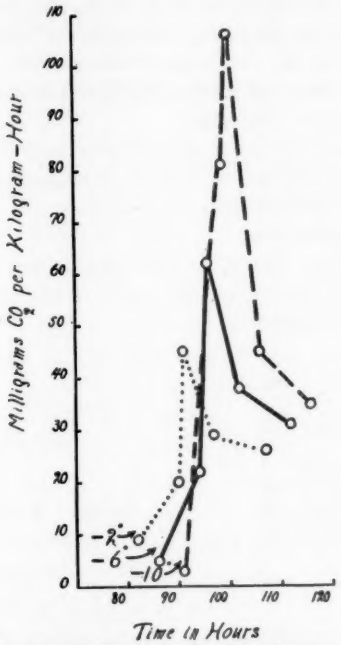

FIG. 3. Output of CO_2 by apple twigs at 6° C. after storage at −10° C., −6° C., and −2° C., respectively, for about 90 hours.

A series of 64 samples was taken from Dec. 21, 1927, to April 15, 1928. After placing in the respiration chamber the CO_2 was swept out continuously and measured every one or two hours until the peak was passed. The data will be presented in two groups. The first involves 8 samples taken during a period of unusual change in temperature. They are presented in fig. 4. The maximum rate of CO_2 production is plotted for each sample. These curves are clearly the mirror images of each other. This means, of course, that the lower the temperature previous to the period at 6° C., the greater is the CO_2 production at that temperature. The second group includes all the samples secured during the whole winter period. The results were analyzed statistically.

The biometrical constants determined are summarized in table II. In this table the letters A, B, C, D, E, F, G, and R have the following significance:

A = the temperature in the orchard at the time of collection.
B = the maximum temperature attained on the day of collection.

C = the minimum temperature attained on the day of collection.

D = the rate of carbon dioxide production (in milligrams per kilogram-hour) during the first hour of aspiration.

E = the maximum rate of carbon dioxide production attained (milligrams per kilogram-hour).

F = the total amount of carbon dioxide produced in the first twenty-four hours of aspiration (milligrams per kilogram).

G = the rate in milligrams per kilogram-hour for the second and third hours of aspiration.

R = the total amount (F) of carbon dioxide produced in twenty-four hours minus the amount produced in the first hour (D) in milligrams per kilogram.

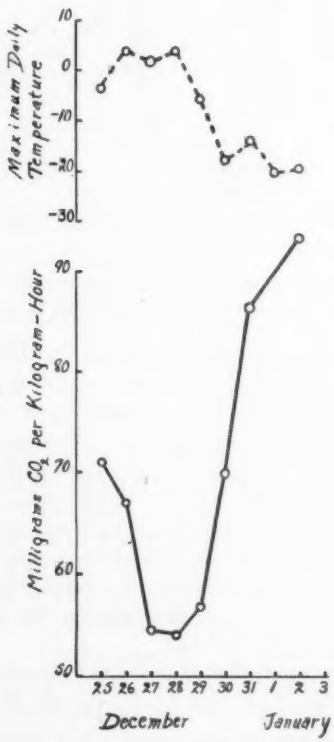

FIG. 4. Effect of orchard temperatures on rate of CO_2 output by apple twigs.

It should be noted that in the case of the temperature values a uniform increment of thirty degrees was added. By this means all temperature readings were raised to positive values.

In the calculation of the biometrical constants reported the following formulae were used:

Mean = $S(X)/N$.

Standard deviation = $\sqrt{S(X)^2/N - \overline{X}^2}$.

Coefficient of correlation = $\dfrac{S(XY)/N - \overline{X}\,\overline{Y}}{s_X s_Y}$.

Coefficient of correlation of a part to the remainder of the whole,* or $r_{XY} = \dfrac{S(XY) - S(X)^2/N - \overline{X}\,\overline{Y}}{s_X s_Y}$.

(Note: This was used only in the case of the correlation coefficient for DR.)

Probable error or mean = $0.6745 \cdot s_X \sqrt{N}$.

Probable error of correlation coefficient = $0.6745 \cdot (1 - r^2)/\sqrt{N}$.

In these formulae the letters have the significance: S = summation, X = the values of one variable, Y = the values of the other variable, N = the number of observation, r = the coefficient, s = the standard deviation, $\overline{X}$ and $\overline{Y}$ the means of the respective variables.

TABLE II

RELATION BETWEEN CO_2 OUTPUT AND PREVIOUS ENVIRONMENTAL CONDITIONS. FOR EXPLANATION OF SYMBOLS SEE TEXT

	MEAN	STANDARD DEVIATION	CORRELATION COEFFICIENT	
A	23.127	7.144	AD	− 0.049 ± 0.084
			AE	− 0.458 ± 0.067
			AF	− 0.424 ± 0.069
B	27.195	7.342	BD	− 0.078 ± 0.084
			BE	− 0.432 ± 0.069
			BF	− 0.399 ± 0.071
C	19.469	7.458	CD	− 0.121 ± 0.083
			CE	− 0.525 ± 0.061
			CF	− 0.490 ± 0.064
D	36.420 ± 1.252	14.764	DE	+ 0.751 ± 0.037
E	53.456 ± 1.140	13.528	GE	+ 0.857 ± 0.022
F	1175.453 ± 24.351	288.809	DR	+ 0.745 ± 0.038

* The writers are indebted for permission to make use of this formula to the originator, Dr. J. A. HARRIS, late Professor of Botany, University of Minnesota.

The results obtained support the conclusion that the rate of respiration is proportional to the increase in temperature to which the twigs are subjected when they are brought from the orchard to the respiration chamber. In addition, another very interesting and important relation is revealed. Thus it is found that a very good correlation exists between the rate of carbon dioxide production in the first few hours of aspiration and the maximum carbon dioxide production attained. The existence of this correlation indicates that in the comparison of the respiration of hardy and tender varieties of apple it is necessary to make determinations during only the first four or five hours.

Of the three temperature values, the minimum gives a better correlation with both the initial and maximum rates, and also with the total production of carbon dioxide, than either the temperature at the time of sampling or the maximum temperature of the day. This was to be expected from the observations above on the influence of extent of elevation of temperature upon carbon dioxide evolution. This is further supported by the fact that the sampling temperature, which was lower than the daily maximum (since the collections were made in the morning), shows a better correlation with both the maximum rate and the total for 24 hours than does the maximum. It is noteworthy also that the correlation of all temperatures with the *maximum rate* of carbon dioxide production is higher than with the initial rate or the total in twenty-four hours. In other words, so far as the effect of temperature is concerned the maximum rate is the most significant measurement. However, as shown by r_{DE} there is a significant relation of the initial to the maximum rate, a relation which it is not unlikely a refinement of technique would show to be more pronounced than is here indicated. Thus, under the experimental conditions adopted, the measurement of the initial rate has been the least accurate of any of the observations made, by reason of the facts that the initial hour was, as a rule, the shortest time interval over which measurements were made, that the amount of carbon dioxide evolved during the first hour was usually less than that given off at any subsequent hour under observation, and that an hour or two was required to bring the twigs to 6° C. In addition, the maximum rate measured is at best only an approximation, especially when the rise and fall in carbon dioxide production is rapid. Early experience showed that a maximum rate could be expected to occur in the vicinity of the 8th to 10th hours. Accordingly, readings were taken more frequently up to this point than later; nevertheless, no claim can be made that the true peak of the curve was more than approximately located.

It is our opinion, therefore, that the actual correlation between the initial rate and the maximum attained is considerably higher than that

actually found, and that a refined technique might make possible the use of a shorter observational period, possibly as low as four hours.

Before offering an explanation of the above results, it might be desirable to summarize them in concise form: (1) When twigs are brought from a lower to a higher temperature for the measurement of CO_2 production, the latter rises to a peak and then declines to a more or less constant value. (2) The lower the previous temperature, the greater is the peak value. (3) The latter principle holds not only for excised twigs in storage, but for twigs on the trees.

Two possible explanations of the above phenomena occur to us. They may be stated as follows: (1) When plant tissues are subjected to the action of low temperatures very considerable changes occur in the tissue-water equilibrium. It is a generally accepted fact that at temperatures below 0° C. ice crystals are formed, not within the individual cells, but in the adjoining intercellular spaces. This withdrawal of water from living tissues must occasion far-reaching disturbances of cellular chemical equilibria and of physical organization. It is accordingly suggested that the apparent increase in carbon dioxide production observed to follow the elevation in temperature of apple twigs from levels below to levels above 0° C. is primarily the result of a temporary stimulation of carbon dioxide production resulting from that derangement of the tissue-water equilibrium. Examples of such low temperature effects upon chemical equilibria within living tissues are the well-known accumulation of sugar in potato tubers, and the increased production of lactic acid in the living muscle of the frog at $-2°$ to $-3°$ C. The postulated stimulation of carbon dioxide production in apple twigs may then be explained as the result of the increased effective concentration of respiratory substrate within the cells following withdrawal of water to the intercellular spaces. Another contributing factor may be increased hydrogen-ion concentration following accumulation of carbon dioxide.

The observed differences between twigs from hardy and from tender varieties of the apple may, on the basis of the above hypothesis, be related directly to the possession by the former of hydrophilic colloids in greater amount, or of more intense imbibitional capacity. On this assumption the water loss from the cells of hardy twigs would be less, and, consequently, the disturbance of normal cellular equilibria correspondingly less, than in the cells of the tender twigs. That is to say, the hardy twigs are more stable or less irritable than twigs of more tender varieties. Further, the return of water to the cells and the resumption of more or less normal metabolism would be more rapid in the case of the former than in that of the latter, a factor conceivably of importance in determining the survival of tissues under rigorous climatic conditions.

(2) Another possibility is that we are dealing here simply with the solution of CO_2 in the twig sap. This gas is increasingly soluble in aqueous media at decreasing temperatures. Therefore, the peak of CO_2 evolution represents the greater solubility of the gas at the previous lower temperature than at the temperature of measurement. Conversely, when measurement is made at a lower temperature than that of the storage, the resultant time curve represents the establishment of an equilibrium between the solubility of CO_2 in the tissue and its diffusion into the outer atmosphere. Evidence favoring this conception is offered in the article following this (**15**). It consists in measuring the dissolved CO_2 of twigs by submerging the latter in boiling alcohol in a closed system and distilling out the CO_2 under vacuum. The CO_2 is completely removed in an hour or so, and it is demonstrable that the amount of the dissolved gas varies inversely with the temperature, and that this is roughly of the same magnitude as that contained in the peak of the curves in this paper. This method had not been devised at the time the measurements in this paper were made; hence, it is utilized here merely as a possible explanation of the observed facts.

Relation of CO_2 production to winter hardiness

A number of varieties of apple known to be of varying degrees of hardiness have been compared in respect to the evolution of CO_2 at 6° C. Only samples collected on the same day and so far as possible subjected to identical conditions subsequent to removal from the tree have been compared directly. Twigs obtained from several different sources and possessing correspondingly variant histories as noted below were utilized.

On January 19, 1928, samples of one-year twigs of Hibernal, Duchess, Charlamoff, McIntosh, Haralson and Jonathan were collected at the University Fruit Breeding Farm. These twigs were taken between the hours of 11 A. M. and 2 P. M., tied in bundles, and kept at a temperature as near as possible to that obtaining in the orchard on that date until received at University Farm, when they were placed in a cold storage room in which the temperature was maintained at 0° C. $\pm$ 2°. Samples were taken for measurement at intervals during the period January 21–31. That the respiratory power of the material did not vary greatly during this period is indicated by the results obtained with Hibernal (table III). The results obtained in the comparison of these different varieties are presented in fig. 5, the curves in which are based either upon single determinations or the average of two or more determinations, as follows:

Beaumont and Hildreth have devised a system of evaluating the amount of winter injury in apple twigs, by the degree of browning in certain specific regions of the twigs. By means of this they have been able to

TABLE III

CONSTANCY OF CARBON DIOXIDE EVOLUTION BY HIBERNAL TWIGS COLLECTED ON JANUARY 19TH, 1928

DATE ANALYZED	NUMBER OF TWIGS	WEIGHT OF TWIGS	CARBON DIOXIDE PER KILOGRAM-HOUR AT 6° C.						
			AT 30 MIN.	AT 3 HRS.	AT 7 HRS.	AT 10 HRS.	AT 15 HRS.	AT 20 HRS.	AT 30 HRS.
			mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Jan. 24	30	137 g.	22	32	39	41	40	39.5	38.0
Jan. 26	40	154 g.	22.5	31	39	41.5	42.5	42.7	.....
Jan. 31	30	135 g.	24	36	38.5	39	39.2	38.5	36.5

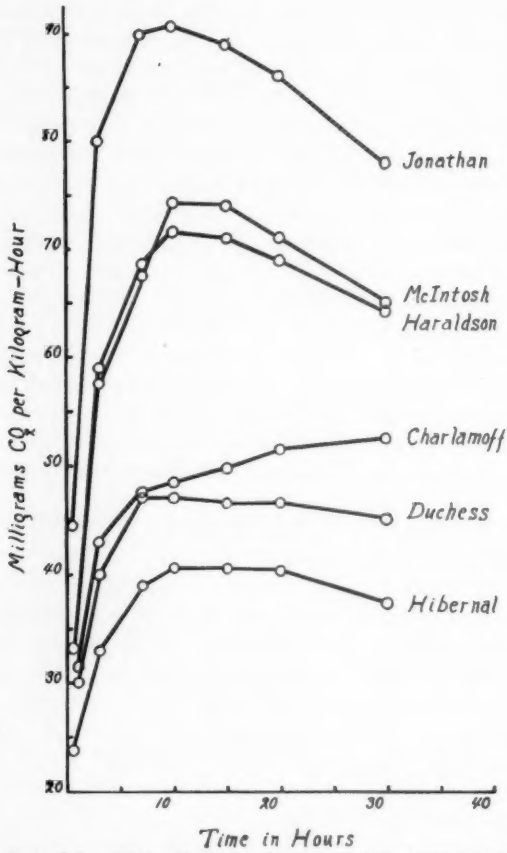

FIG. 5. CO_2 output of 6 varieties of apple twigs of varying degrees of winter hardiness.

TABLE IV

DETERMINATIONS USED IN FIG. 5

VARIETY	NO. SAMPLES	DATE DETERMINED	AVERAGE WEIGHT OF SAMPLES
Haralson	3	Jan. 24, 26, and 31	129
Duchess	1	Jan. 21	126
Charlamoff	2	Jan. 21 and 24	131
McIntosh	2	Jan. 27 and 29	114
Haralson	3	Jan. 26, 27 and 29	91
Jonathan	1	Jan. 31	136

assign definite numerical hardiness values by which the varieties may be compared. Table V contains their values.* The lowest values indicate least browning and hence greatest hardiness.

It will be seen that the order of magnitude of CO_2 production in fig. 5 is the same as the order of hardiness for these 6 varieties. Thus the hardiest varieties evolve the least CO_2 under the conditions of measurement, and the tenderest evolve the most. Not only is the serial order the same, but the quantitative relations are rather regular; thus the hardiness values of the 6 varieties in fig. 5 from bottom to top are 6, 8, 10, 12, 16, and 24.

TABLE V

BEAUMONT AND HILDRETH'S HARDINESS VALUES FOR APPLE VARIETIES

VARIETY	AVERAGE BROWNING FOR 4 YEARS— 1924–1928	AVERAGE BROWNING FOR 3 YEARS— 1924–1927
Dolgo	2.0	2.3
Hibernal	7.0	6.0
Oldenburg (Duchess)	7.0	7.7
Charlamoff	.....	10.0
Patten	11.8	11.0
Haralson	14.5	12.0
Wealthy	15.5	12.7
Anisim	.....	14.0
McIntosh	21.2	15.7
Minnehaha	20.5	17.0
Fameuse	22.2	16.3
Cortland	.....	18.3
Salome	.....	19.3
Delicious	25.0	20.0
Wolf River	26.8	21.0
Winesap	29.0	22.0
Jonathan	28.5	23.7
Ben Davis	28.5	24.0
Sugar Loaf	.....	24.7
King David	.....	25.7
Lansingburg	.....	25.7
Yellow Belleflower	.....	30.3
Tompkins King	.....	36.3

These results are contrary to the preliminary measurements of WILLAMAN and BEAUMONT on Charlamoff and Delicious (**13**), in which the hardier seemed to emit more CO_2 than the tender. The explanation is that at the time those measurements were made we did not realize the very great effect

* Although these values have not been hitherto published, a description of the method of arriving at the values is given by B. H. WILSON in Sci. Agr. **10**: 598–606. 1930.

of the previous temperature. The two varieties were measured alternately and no attention paid to the temperature previous to each measurement. We can see now that this fact could completely jeopardize any varietal differences.

Since examination showed that considerable winter injury had already occurred in certain of the samples reported on above, as indicated by the "browning test," particularly in McIntosh, Haralson, and Jonathan, it appeared possible that the increased CO_2 production shown by these varieties over that of the more hardy sorts might be the result of this winter injury rather than due to any inherent difference in respiratory power. Accordingly, samples of Red Duchess and Delicious, in which practically no frost injury could be detected, were obtained from the tubbed breeding stock in the cold storage cellar at the Fruit-breeding Farm. These samples were taken on February 10th, held in the cold cellar of the Horticultural Build-

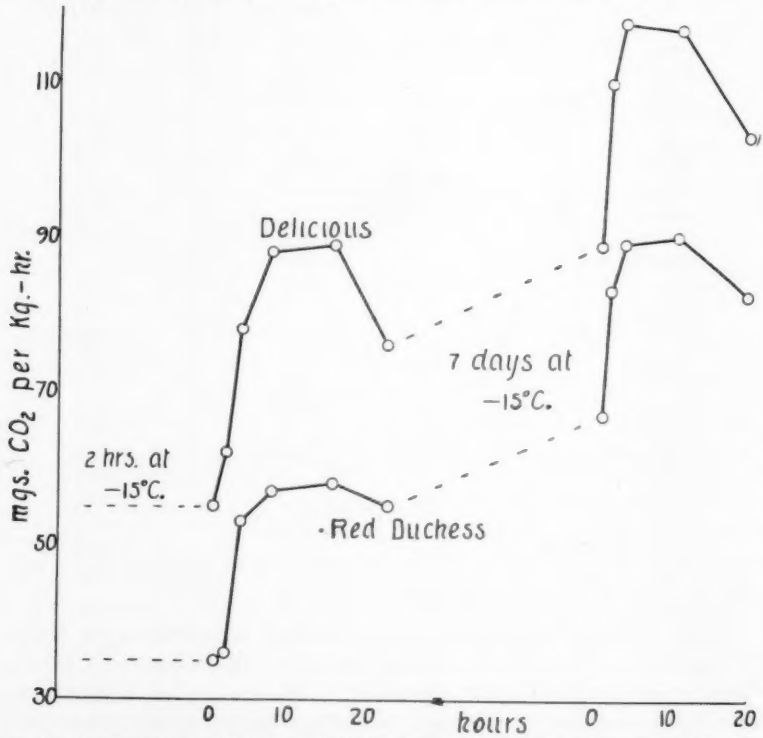

FIG. 6. Accumulation of CO_2 in apple twigs stored at $-15°$ C. These curves also illustrate a varietal difference due to hardiness, Duchess being hardier.

ing at University Farm over-night at a temperature of about 5° C., and then, on the morning of February 11th, subjected to a temperature of – 15° C. for two hours before aspiration was commenced. The temperature changes occurring subsequent to removal of these twigs from the long-continued even temperature of the storage cellar at the Fruit Farm probably account for the relative high rate of carbon dioxide production found in both these samples. No more of this material was available. Consequently, after twenty-four hours' aspiration the twigs were replaced in the storage room at – 15° C., where they were held for seven days. At the end of this time they were again aspirated at 6° C. for a second twenty-four hour period. The results obtained are shown in fig. 6. It will be noted that the respiratory level has been raised by holding at – 15° C. in the case of both varieties, but that the *relative* difference between the hardy Duchess and the tender Delicious has not been materially altered. It is therefore considered that the differences shown in fig. 6, as well as in fig. 7, are due to inherent varietal qualities rather than to low temperature injury.

Further data respecting the relative rates of carbon dioxide production in hardy and tender varieties were obtained on materials of a more restricted range in hardiness, none of which showed appreciable injury. Such was the case for the comparison of Patten with Duchess of Oldenberg, and Florence with Hibernal, the former variety in each instance being the less hardy. Samples were taken on 4 different dates for the first pair and on 5 for the second. The data for the various samples were averaged, and

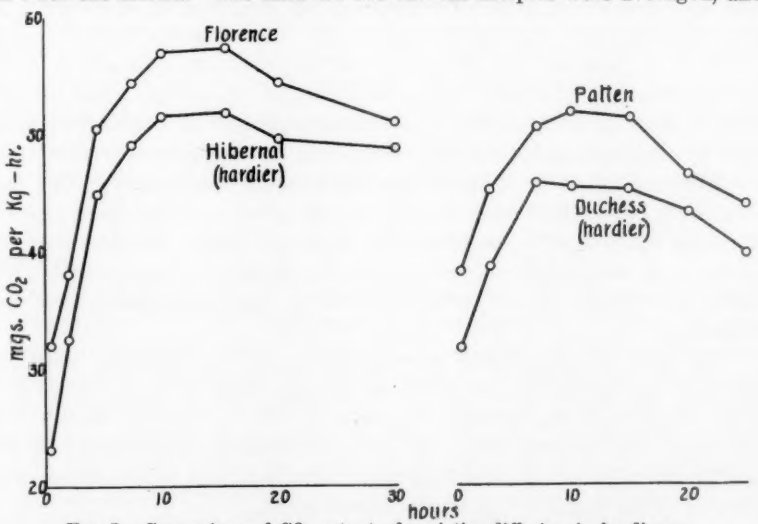

FIG. 7. Comparison of CO_2 output of varieties differing in hardiness.

are presented in fig. 7. The mean difference with its probable error, the standard deviation, and the deviation from a random distribution were calculated for these data, and found to be as follows:

TABLE VI

STATISTICAL STUDY OF DIFFERENCES BETWEEN NON-HARDY AND HARDY APPLES

VARIETIES	MEAN DIFFERENCE	STANDARD DEVIATION OF DIFFERENCE	DEVIATION FROM RANDOM DISTRIBUTION
Florence–Hibernal	$\pm$ 5.4903 $\pm$ 0.6494	5.3605	+ 9.5 $\pm$ 1.8777
Patten–Oldenburg	$\pm$ 5.9425 $\pm$ 0.4088	4.0408	+ 18.0 $\pm$ 2.2483

For the first two varieties the mean difference is thus seen to be more than eight times its probable error, while the deviation from a random distribution is more than five times as great as the probable error. In the case of Patten and Duchess the differences are even more significant, the corresponding values being nearly fifteen and more than eight times their probable errors, respectively.

We were able to make still another comparison of varieties during the winter of 1928–9. Eleven varieties were secured at the University of Minnesota Fruit Breeding Farm on Dec. 28, stored at – 15° C., and analyzed during the next few days. The data secured are given in table VII. Before placing in the respiration chamber the twigs in series A were kept at 20–23° C. Those in series B were placed in the chamber directly from the storage chamber. Because of the small number of samples it was not considered feasible to calculate the ordinary coefficient of correlation, hence that by rank was used, according to the formula $r = 1 - 6\ (SD^2_k)/n^3 - n$.

Although in series A the agreement between hardiness and CO_2 production is only fair, that in series B is very good, and they both indicate the same relation in the samples of the previous winter. We therefore feel justified in concluding in general that the hardier an apple variety, the lower its intensity of CO_2 production under the experimental conditions described.

The question immediately arises as to whether the solubility of CO_2 in the twig sap can be invoked here also in explanation of these facts. Evidence is presented in the following paper by WILLAMAN and BROWN (**15**) that such may be the case: these varieties differ in their content of dissolved CO_2 in the same order as their hardiness, and in quantities comparable to the peaks in CO_2 curves shown above.

TABLE VII

CO_2 OUTPUT OF APPLE TWIGS AT 6° C. DURING FIRST SIX HOURS

VARIETY	HARDINESS VALUE*	SERIAL ORDER OF HARDINESS	A		B	
			THAWED BEFORE PLACING IN THERMOSTAT**		NOT THAWED	
			CO_2 PER KG.	SERIAL ORDER	CO_2 PER KG.	SERIAL ORDER
			mg.		*mg.*	
Hibernal	6	1	88	3	106	2
Duchess	8	2	80	1	98	1
Patten	11	3	105	8	133	4
Haralson	12	4	97	7	146	7
Wealthy	13	5	90	5	138	6
McIntosh	16	6	91	6	137	5
Fameuse	16	7	88	4	126	3
Wolf River	21	8	81	2	154	8
Jonathan	24	9	128	10	175	9
Sugar Loaf	25	10	119	9	212	10
Lyman Perfect***			93		.....	

* BEAUMONT and HILDRETH.

** Kept in laboratory for 30 minutes.

*** Not listed by BEAUMONT and HILDRETH.

Coefficient of correlation (by rank) between tenderness and CO_2 output:—

$r_A = +0.478 \pm 0.172$

$r_B = +0.818 \pm 0.074$

CHANGES IN COMPOSITION OF EXCISED TWIGS

It seemed desirable to determine to what extent chemical changes were occurring in the twigs under the experimental conditions used. Analytical results on a number of samples under various storage conditions are given in table VIII. The averages of the changes in composition at each temperature were calculated to a basis of 10 day periods, and are given in table IX.

No striking changes in composition occurred. Nevertheless, certain tendencies are apparent, which may be summarized as follows: (1) The moisture losses are not great. (2) With aspiration, there is a slight gain in starch and a loss in sugars. (3) Without aspiration there is not much change in starch, and a gain in sugars. (4) In other words, the accumulation of CO_2 in the air around the twigs, and hence its presumed accumulation in the tissues, causes an accumulation of sugar in the tissue, either because of reduced respiration, or because of a changed equilibrium between starch and sugar.

TABLE VIII

CHANGES IN COMPOSITION OF EXCISED TWIGS DURING STORAGE AT LOW TEMPERATURES

DATE	MOISTURE	REDUCING SUGARS		TOTAL SUGARS		ACID-HYDROLYZABLE "STARCH"		REMARKS
		FRESH	DRY	FRESH	DRY	FRESH	DRY	
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
Feb. 3	46.0	1.52	2.82	5.47	10.93	10.8	20.0	At time of collection
	40.7	2.12	3.58	6.67	11.26	12.0	20.2	After 18 days without aspiration at −10°
	46.4	1.28	2.39	4.40	8.22	11.5	21.5	After 21 days aspiration at −2°
	41.2	1.32	2.25	4.51	7.67	12.6	21.4	After 21 days aspiration at −6°
	45.4	1.31	2.40	4.27	7.83	12.0	21.5	After 21 days aspiration at −10°
Feb. 26	43.9	1.26	2.25	4.19	7.47	12.3	21.9	At time of collection
	42.3	1.43	2.48	4.80	8.33	12.7	22.0	After 6 days without aspiration at −6°
	42.7	1.40	2.45	4.88	8.53	12.6	22.1	After 6 days without aspiration at −10°
	43.6	0.99	1.76	3.55	6.30	12.5	22.2	After 30 days aspiration at −2°
	41.9	1.28	2.20	4.58	7.88	13.0	22.4	After 30 days aspiration at −6°
	44.5	1.14	2.06	3.97	7.16	12.0	21.7	After 30 days aspiration at −10°

TABLE VIII—(*Continued*)

Date	Moisture	Reducing sugars		Total sugars		Acid-hydrolyzable "starch"		Remarks
		Fresh	Dry	Fresh	Dry	Fresh	Dry	
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	
March 29	47.9	1.28	2.46	4.43	8.51	11.1	21.4	At time of collection
	45.2	1.46	2.66	5.30	9.90	11.5	21.1	After 4 days without aspiration at −2°
	46.8	1.43	2.69	4.93	9.28	11.5	21.6	After 4 days without aspiration at −10°
	45.2	1.15	2.10	4.68	8.55	12.0	22.0	After 8 days aspiration at −2°
	47.0	1.17	2.21	4.27	8.06	11.6	22.0	After 8 days aspiration at −10°
April 6	48.9	1.12	2.19	4.05	7.93	11.5	22.5	At time of collection
	44.6	1.58	2.85	5.97	10.78	11.1	20.1	After 8 days without aspiration at −2°
	47.2	1.08	2.05	4.68	8.86	10.8	20.5	After 8 days without aspiration at −10°
April 16	45.4	1.20	2.20	3.58	6.57	10.1	18.5	At time of collection
	50.0	1.89	3.79	7.17	14.36	11.2	22.4	After 13 days without aspiration at −2°
	49.5	1.22	2.42	4.64	9.20	10.8	21.5	After 13 days without aspiration at −10°

TABLE IX

CHANGE IN COMPOSITION OF APPLE TWIGS PER 10 DAY PERIODS UNDER VARIOUS STORAGE CONDITIONS

CONSTITUENTS	DRY MATTER BASIS					
	WITH ASPIRATION			WITHOUT ASPIRATION		
	−2° C.	−6° C.	−10° C.	−2° C.	−6° C.	−10° C.
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Starch	+0.54	+0.44	+0.47	−0.30	+0.11	+0.16
Reducing sugars	−0.27	−0.15	−0.19	+0.85	+0.38	+0.26
Total sugars	−0.43	−0.53	−0.69	+3.01	+0.86	+1.46
Moisture	−1.50	−1.50	−0.43	−2.70	−2.50	−1.20

These results cannot be emphasized strongly, because of the limited number of samples and the rather small differences in composition. Nevertheless, they are suggestive and are included here partly to augment the very sparse published analyses of apple twigs.

Discussion

It has been shown that storage of apple twigs at lower temperatures, followed by their elevation to a higher temperature, gives rise temporarily to a considerable increase in rate of CO_2 output. Furthermore, the rate at the higher temperature is greater, the lower the previous temperature of storage. The same facts obtain when twigs are not taken from storage, but measured immediately after taking from the tree. It has also been shown that the hardier varieties respond to such variations in temperature to a less degree than the tenderer. We have shown in another paper that the CO_2 dissolved in the twig tissue can probably account for most of this temporary flush, and that the quantity of this dissolved CO_2 is proportional to the tenderness of the variety.

These findings agree with many of the observations found in the literature of plant respiration. In 1885 DEHÉRAIN and MAQUENNE (**2**) first called attention to the CO_2 dissolved in plant sap and to its possible effect on respiration measurements. Later MAQUENNE (**6**) attempted to remove this CO_2 by evacuation, but was only partly successful. SIMON (**12**) has measured the CO_2 production of excised twigs of beech, red oak, basswood, and horse chestnut. The twigs were brought from various outdoor temperatures to 22.5° C. for measurement, and SIMON noted that the lower the previous temperature, the greater was the production of CO_2 during

the early part of the measurement period. This effect was so pronounced that he extended his measurements over a period of seven days in order to avoid that temporary flush.

Increasing the temperature within the range of normal growth has frequently been observed to produce a temporary increase in CO_2 evolution. A very definite effect of this nature was early noted by MÜLLER-THURGAU (**8**) in potato tubers, and has since been reported for many other types of tissue, as, for example, etiolated shoots of *Vicia faba* (PALLADIN, **10**), gladiolus bulb (ZALESKI, **16**), and possibly sweet potato tubers (JOHNSTONE, **5**) and bananas (OLNEY, **9**).

In the case of the sweet potatoes the tubers were stored at 15–20° C. for some time and then brought to 25° C. for CO_2 measurement. The curves show a peak at about the tenth day, followed by a decline to a constant level for another 20 or 30 days. The author says, "The slight increase in respiration shown by the curves on the fourth to seventh days is undoubtedly due to transferring the sweet potatoes to the higher temperature for respiration determination, but cured sweet potatoes do not respond so readily to such a change as uncured." This statement does not, of course, explain the temporary flush of CO_2. We cannot readily see how this peak could be due to dissolved CO_2 accumulated at the lower temperature, because it requires seven to ten days for dissipation. In the case of the bananas the respiration curves have a shape very similar to those of apple twigs; but again the peak comes during the second to fourth days. It is possible that in each case we are dealing with the slow evolution of dissolved CO_2, and that the thickness of the tissue, its wateriness and its solvent power for CO_2 determine the time required for an equilibrium to be established at a higher temperature. It might be mentioned here that, when twigs are submerged in boiling alcohol and evacuated, an hour and a half is required for complete removal of the dissolved CO_2; hence 5 to 10 days might be a plausible period for this purpose in a tissue like sweet-potato tubers, with only the normal circumstances for diffusion of the gas.

Respiratory activity in winter cereals has been more recently studied by GOVOROV (**4**) and by MARTIN (**7**). The former found that in winter wheat and rye the amount of CO_2 liberated was 300 and 800 mg. per hour per kilo of dry weight at 3° C. He also found that spring wheat and spring rye, under the same conditions, gave off considerably larger quantities of carbon dioxide, namely, 1800 and 1700 mg. respectively. It should be noted that the roots of these plants were removed prior to the determination of the respiratory rate, and also that during the twelve hours previous to this operation, the plants had been maintained at 0° C. The stimulation resulting from the wounding incident to the removal of the roots, and from the rise in temperature, may therefore be assumed to be, in part at

least, responsible for the fact that these results are considerably higher than those reported by MARTIN for similar material. The latter has determined the respiration of a number of varieties of winter and spring wheat and of rye at still lower temperatures. His results may be summarized as follows:

	Milligrams CO_2 per kilogram of dry weight per hour		
	At 0°	At −5°	At −10°
Wheat	182.5–259.3	141.3–188.6	50.3–127.0
Rye	516.9	165.8	70.8

In accordance with the results of GOVOROV, the hardier varieties were found to evolve less carbon dioxide than those less resistant to low temperatures, particularly at −10°. The relation between wheat and rye at 0° is also similar to that found by GOVOROV.

It should be noted that MARTIN used the same plants for the determination of the respiratory activity at the different temperatures and proceeded from the higher to the lower levels, allowing 12 hours for accommodation to the new temperature. This is important, since there is much evidence that the direction in which the temperature alteration occurs may cause appreciable differences in the results obtained at the new level.

Although the balance of the evidence is in favor of the view that increasing the temperature of plants brings about stimulation of the rate of respiration, there is, however, some contrary evidence. ZIEGENBEIN (**17**), for example, found no stimulation in respiration on raising the temperature of seedlings of vetch and lupine from 15–20 to 30° C. He used the continuous aspiration method and apparently ample time was allowed for the temperature equilibrum of the tissues with the surrounding atmosphere to become established at the different temperatures.

BLANC (**1**), also, has concluded that, in the case of etiolated shoots of *Vicia faba*, bean seedlings, and young leaves of rye, the respiratory transition is gradual from one temperature to another and without stimulative effects due either to rising or falling temperature.

Nevertheless, we are confident that, when plant tissue is brought from a lower to a higher temperature for the measurement of its CO_2 production, the resultant curve will typically not assume a level until after a preliminary peak has been passed. We further believe that this peak is largely explainable by the lesser solubility of the CO_2 at the higher temperature; and that the CO_2 of the peak of the curve represents that which was produced by respiration at some time previous to that of the measurement.

Summary and conclusions

1. It has been shown that wound respiration in excised apple twigs is a small and relatively constant fraction of the total respiration.

2. The rate of CO_2 production as measured at any temperature is conditioned by the previous temperature environment of the twigs. If the previous temperature has been higher, a constant level of CO_2 emission is gradually assumed. If the previous temperature has been lower, there is a peak of CO_2 evolution for several hours, after which a level is gradually attained.

3. The lower the previous temperature, the higher is the peak amount of CO_2. This was proved both by twigs kept in storage before measuring the CO_2 output, and by twigs taken directly from the orchard at various temperatures throughout the winter.

4. It is suggested that this temporary excess of CO_2 represents that which was produced during the previous period but which remained dissolved in the twig sap, and which diffuses out of the twigs at the higher temperatures because it then becomes less soluble.

5. Hardier varieties of apples show a lower peak of CO_2 evolution than do the tenderer. In fact, the order of increasing tenderness among 11 varieties coincides almost exactly to that of increasing CO_2 during the peak.

6. Twigs stored at $-2°$ C., $-6°$ C., and $-10°$ C. for periods up to 30 days with and without aspiration of the surrounding air did not show striking changes in chemical composition. However, without aspiration there seems to be slight gain in sugar; and with aspiration there seems to be a slight gain in starch and loss in sugars.

7. It is emphasized that in the measurement of the respiratory rate of any plant tissue the effect of dissolved CO_2, and hence the previous temperature history of the material, must be kept in mind.

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CARBON DIOXIDE DISSOLVED IN PLANT SAP AND ITS EFFECT ON RESPIRATION MEASUREMENTS[1]

J. J. WILLAMAN[2] AND WILLIAM R. BROWN

(WITH THREE FIGURES)

We have undertaken to demonstrate that certain phenomena of "respiration" in plants are not due to CO_2 which is produced during the period of measurement, but are due to the CO_2 which is dissolved in the sap of the tissues. The following paper contains our method for measuring this dissolved CO_2 as well as experiments which lead us to certain conclusions as to its significance.

In the preceding paper (**3**) it has been shown that storage of apple twigs at a lower temperature, followed by their elevation to a higher temperature, gives rise temporarily to a considerable increase in rate of CO_2 output. Furthermore, the rate at the higher temperature is greater, the lower the previous temperature of storage. The same facts obtain when the twigs are taken directly from the trees: the rate of CO_2 output varies inversely with the orchard temperature at the time of collecting the twigs.

CO_2 has, of course, a solubility in aqueous media that varies inversely with the temperature. Presumably, plant sap as a medium is no exception. This principle can explain the above case. Thus, when twigs are raised to a higher temperature for measurements of CO_2, the temporary flush which is observed indicates not a greater production of CO_2 at that time, but represents CO_2 which was previously produced and which remained dissolved in the tissue sap. After a certain period at the higher temperature a new equilibrium is established and from that time on the output of CO_2 is more or less constant for that temperature.

WILLAMAN and BEAUMONT (**11**) recently published some results on the effect of allowing CO_2 to accumulate in the atmosphere of a respiration chamber. They state: "When the accumulated CO_2 is removed, the rate of its production immediately assumes a far higher value; and the magnitude of this increased value is possibly proportional to the amount of CO_2 previously accumulated (in the chamber). It is a matter of several hours before the rate assumes a constant value." They introduced some evidence to show that the explanation of this phenomenon lay at least partly in the fact that the dissolved CO_2 increased the hydrion concentration of the tissue and that this stimulated respiration. We believe now that another

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[2] Now at Röhm and Haas Co., Inc., Bristol, Pa.

part of the explanation lies in the accumulation of CO_2 in the tissues, because of the greater partial pressure of CO_2 in the atmosphere surrounding the twigs. When this partial pressure is reduced, the dissolved CO_2 diffuses out of the twigs and causes the apparent temporary increase in respiratory rate which is always observed. Ultimately, a new equilibrium is established, and the output of CO_2 becomes constant, unless another period of accumulating CO_2 in the chamber intervenes.

The difference in the above two cases is that in the latter the temperature was constant and the CO_2 accumulated in the tissue because it was also accumulating in the atmosphere around the tissue; while in the former case the accumulation in the tissue was only relative because of the difference in solubility of the CO_2 at the different temperatures.

In the preceding paper (**3**) data are submitted which indicate that those varieties of apples which possessed the greatest winter hardiness seemed to have the lowest rate of CO_2 output, while the tenderer varieties had the highest rate. In the present paper we show that the varietal differences in apparent CO_2 output are paralleled by differences in the amount of dissolved CO_2. The hardier varieties contain less, and the tenderer more, dissolved CO_2.

Although we have only a limited number of determinations of dissolved CO_2 at the present time, they are rather definite in their trend, and it seemed best to publish them simultaneously with those in the preceding paper, because we feel that they partly explain the findings in the latter paper.

In 1885, DEHÉRAIN and MAQUENNE (**2**) first called attention to the CO_2 dissolved in plant sap and to its possible effect on respiration measurements. Later MAQUENNE (**5**) attempted to remove this CO_2 by evacuation, but was only partly successful. SIMON (**9**) has measured the CO_2 production by excised twigs of beech, red oak, basswood and horse chestnut. The twigs were brought from various outdoor temperatures to 22.5° C. for measurement, and SIMON noted that the lower the previous temperature the greater was the production of CO_2 during the early part of the measurement period. This effect was so pronounced that he extended his measurements over a period of seven days in order to avoid that temporary flush. Increasing the temperature has frequently been observed to produce a temporary increase in CO_2 evolution. A very definite effect of this nature was early noted by MÜLLER-THURGAU (**6**) in potato tubers, and has since been reported for etiolated shoots of *Vicia faba* by PALLADIN, (**8**), gladiolus bulbs by ZALENSKI, (**12**), sweet potatoes by JOHNSTONE, (**4**), and bananas by OLNEY (**7**).

Apparatus and method

The apparatus as finally used is illustrated in fig. 1, and is largely self-explanatory. The container D can be of glass or of copper. The procedure

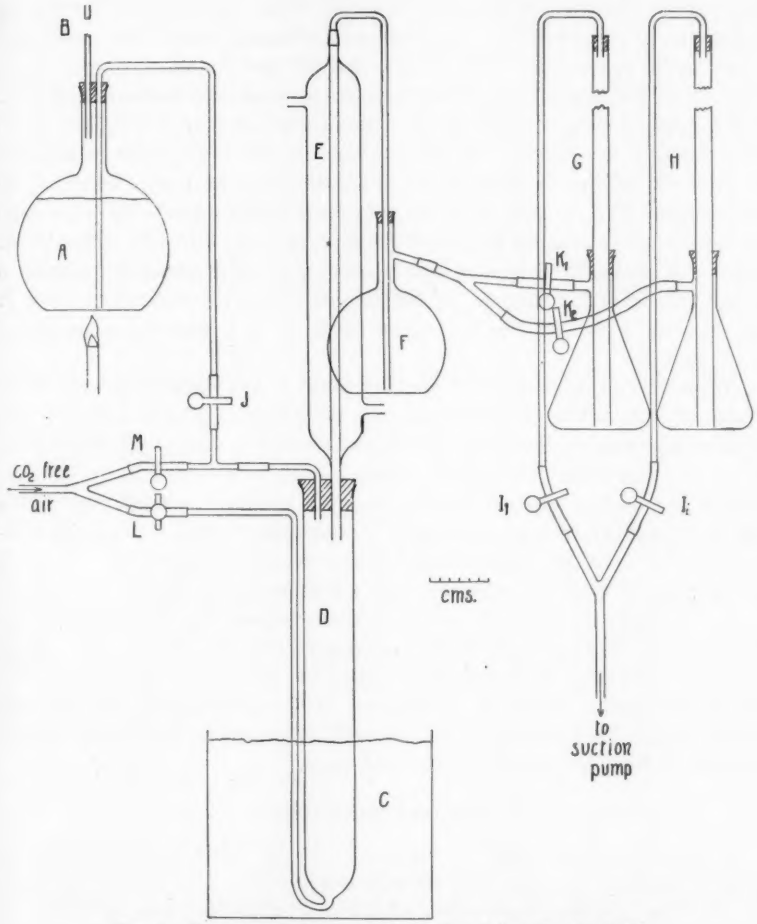

FIG. 1. Diagram of apparatus for determining dissolved CO_2.

is as follows: The bead towers (1) are charged with alkali, usually 25 cc. of 0.2N NaOH, and clamps I_1 and I_2 are closed. About 400 cc. of 95 per cent. alcohol is boiled in A for about 5 minutes to free it of CO_2. The water in bath C is heated to about 90° C. With the apparatus thus in readiness, the sample of twigs, in apples about 150 to 170 gm., is placed in the tube D, with the cut ends up. This must be done at the place where the twigs have been, either in the orchard or in storage, so that the twigs shall not be exposed to a higher or lower temperature until they are in the tube and

stoppered. The small outlet tube is closed with a piece of rubber tubing and a clamp. The tubes are then taken immediately to the laboratory and placed in the apparatus. The pump is started, and clamps J, K_1 and I_1 are opened. J is closed as soon as the twigs are covered with the boiling alcohol. Screw-clamp L is opened enough to allow a slow stream of CO_2-free air to pass through the alcohol. A proper balance will have to be established between the partial vacuum in the apparatus and the temperature of the water bath. For the first 30 or 40 minutes it is desirable to have the temperature of the alcohol as high as possible so as to kill the cells of the twigs. Later it is desirable to have a higher vacuum so as to assist the expulsion of the CO_2 from the tissues. If the alcohol froths too violently, clamp M can be opened momentarily. It is useful to have a manometer connected to the system.

When there is reason to believe that tower G has received the allowable charge of CO_2, tower H is shunted into the circuit. This is done by first opening K_2, then gradually opening I_2 and closing I_1, and finally closing K_1. After one and a half hours, the towers should be changed every 15 min. until it is apparent that all the CO_2 has been obtained. Furthermore, the time required for each material may be determined, and this period arbitrarily used in all subsequent cases without changing the towers. We have found that 120 minutes suffice for apple and cherry twigs.

In titrating, the tower is removed from its flask, the beads allowed to slide into the flask, and the tower rinsed with CO_2-free water. The alkali is then back-titrated with standard acid in the presence of the beads. We prefer the double indicator of SIMPSON (**10**), containing cresol red and thymol blue. This gives the CO_2 in one titration and avoids the use of any barium for the precipitation of the carbonate.

Experimental results

A typical determination of dissolved CO_2 by the method described is given in table I. In most cases the exhaustion of the CO_2 was completed in 105 minutes. At the present time we are finding that for most materials we can use a single titration at the end of 120 minutes.

On Feb. 15, 1929, one-year old twigs of Duchess were collected at University Farm and divided into 9 lots. Two were analyzed immediately, 3 were stored at 4–6° C., 2 at −15° C. and 2 at −22° C. They were laid on open shelves in the storage rooms. At intervals they were analyzed for dissolved CO_2. The results are shown in fig. 2.

One immediately obvious fact is that the amount of the contained CO_2 was inversely proportional to the temperature of storage. This is brought out more strikingly when the amounts of CO_2 are plotted against tempera-

TABLE I

REPRESENTATIVE DETERMINATION OF DISSOLVED CO_2
SAMPLE = 92.0 GM. OF DUCHESS TWIGS

TIME	CO_2
minutes	*mg.*
15	1.89
30	8.45
45	6.93
60	3.15
75	2.39
90	1.38
105	0.88
120	0.13
Total	25.20

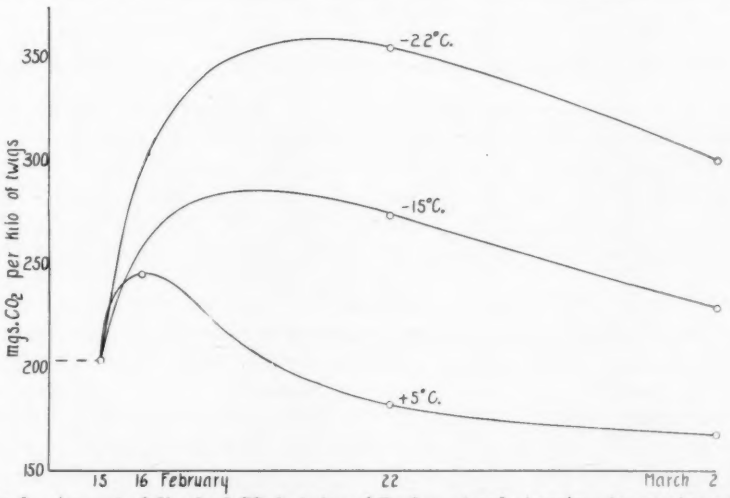

FIG. 2. Amount of dissolved CO_2 in twigs of Duchess stored at various temperatures for various lengths of time.

ture, as in fig. 3. These curves indicate a rapidly increasing solubility of CO_2 in sap at low temperatures.

Fig. 2 also shows that during the period of the experiment the amount of CO_2 at all three temperatures declined. This probably indicates some change in the solution properties of the sap towards CO_2.

On Dec. 28, 1928, samples of twigs of several varieties of apples were collected at the University of Minnesota Fruit Breeding Farm at Excelsior.

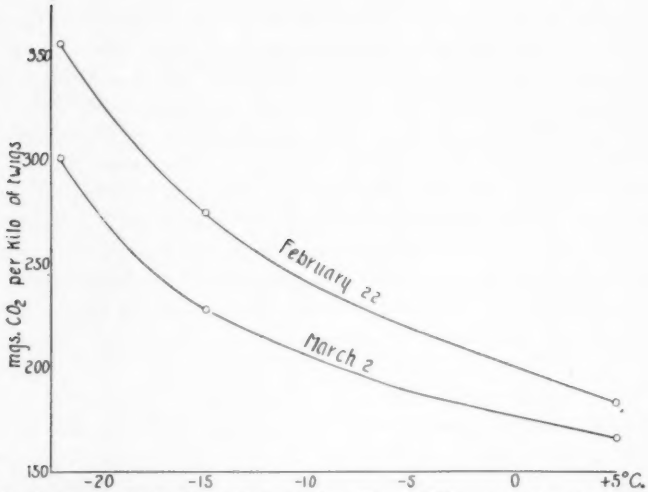

FIG. 3. Same data as in fig. 2, but showing the solubility at various temperatures.

They were stored on open shelves at – 15° C. Parts of these samples were used for the respiration measurements recorded in the preceding paper (**3**). In February some of them were still available; and although they were rather old by this time we decided to measure their dissolved CO_2. The results are given in table II. Although there were only five varieties for which BEAUMONT and HILDRETH have hardiness values, and although they

TABLE II

RELATION OF DISSOLVED CO_2 IN APPLE VARIETIES TO THEIR WINTER HARDINESS

VARIETY	HARDINESS*	CO_2 PER KILO
Duchess	8	202
Patten Greening	11	279
Wealthy	13	315
Wolf River	21	434
Sugar Loaf	25	384
Lyman Perfect	**	276

* According to BEAUMONT and HILDRETH'S scale (3, p. 523), the larger the number, the greater is the tenderness.

** Not listed by BEAUMONT and HILDRETH.

had been off the tree for seven weeks, a striking relation is seen between the dissolved CO_2 and the hardiness. Only one variety, Sugar Loaf, is an exception, and this is only one position out of place. In other words, the hardier varieties contain less CO_2 in their sap, and the tenderer contain more.

In the preceding paper it was shown that the hardier varieties give off less CO_2 than the tenderer. Thus the corollary to these facts is that a lower output of CO_2 is accompanied by a lower solvent power for the gas. The explanation for this relation must involve some of the fundamental properties of the twig protoplasm.

Summary and conclusions

A method has been devised for determining the CO_2 dissolved in twig sap. It consists essentially in submerging the twigs in boiling 95 per cent. alcohol in a closed container, and then removing the CO_2 with reduced pressure. The CO_2 is caught in standard alkali and titrated.

The amount of CO_2 contained in apple twigs is of the magnitude of 150 to 250 mg. per kilo at 0° C. The amount varies inversely with the temperature, and may be as high as 360 mg. at – 22° C.

We believe that this dissolved CO_2 may explain at least three phenomena of plant "respiration" measurements, meaning by the latter term the measurement of the CO_2 given off by the plant tissue under stated circumstances. These phenomena are as follows:

(1) When apple twigs are raised from a lower to a higher temperature, the output of CO_2 at the higher temperature is temporarily increased above the level that it subsequently attains at that temperature. The effect is greater, the lower the previous temperature. The temporary flush is caused, we believe, by the lower solubility of the CO_2 at the higher temperature.

(2) When plant tissue (twigs, potato tubers, and wheat grain have so far been observed) is stored in a closed chamber and the CO_2 allowed to accumulate, and then the CO_2 swept out and the rate of its output by the tissue measured, this rate is found to rise to a temporary high value. Ultimately, this rate subsides to a lower level characteristic of the temperature used. We believe that this flush may indicate an actual increase in rate of production of CO_2, occasioned by the higher acidity of the sap during the accumulation of CO_2. The peak of CO_2 output is probably also accounted for partly by the diffusion of previously formed CO_2 which was held in solution because of the higher partial pressure of CO_2 in the atmosphere surrounding the tissue.

(3) Those varieties of apples which possess the greatest winter hardiness show the lowest output of CO_2. We have found that they also contain

the lowest amount of dissolved CO_2. What the causal connection is between the two is not known at present.

The generality which we deduce from these results is that in plant respiration studies a careful distinction must be made between the *production* of CO_2 and its *output*. The one may or may not be the same as the other, due to the various factors which affect the amount of dissolved CO_2. The factors known at present are temperature, partial pressure of CO_2 in the atmosphere around the tissue, and the kind of tissue.

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INFLUENCE OF LEAFAGE REMOVAL ON ANATOMICAL STRUCTURE OF ROOTS OF *STIPA PULCHRA* AND *BROMUS HORDEACEUS*

KENNETH W. PARKER[1] AND ARTHUR W. SAMPSON[2]

(WITH FOUR FIGURES)

Historical

Numerous investigations have been reported to show the influence of the reduction of chlorophyll-bearing tissue, as in the pruning of fruit trees or in the cutting or grazing of fodder and forage plants, on the subsequent yields. There has been little serious investigation to show the effect of harvesting on the physiological responses of the plant, and more especially on the anatomical structure of the roots.

PRIESTLEY and EVERSHED (**8**) have shown that root growth is periodic and seasonal in many species remotely related taxonomically. Removal of succulent leafage, however, tends clearly to unbalance, or actually to destroy, any rhythm that might otherwise exist between top and root growth. Moreover, defoliation is definitely correlated with the extent of root growth, such as may be expressed in dry weight or in volume of root expansion. Only a slight reduction in the area of active chlorophyll-bearing parts typically causes a correspondent slowing down in root growth. If the decreased elaboration of organic materials is further intensified by herbage removal, root growth may cease entirely. Defoliation at frequent intervals of the plants studied finally caused destruction of the root system as a whole, and death of the plant. In studies by Sampson (**9**) plots of *Festuca viridula* in Oregon were harvested three times annually for three successive years. Root and aerial growth, including size of tufts, decreased annually during this treatment.

SAMPSON and MALMSTEN (**10**) working with many species of bunch grasses and other plants, found that the removal of herbage four to five times in a season, at approximately monthly intervals, resulted in a sharp decline in yield and a marked shortage of life of the vegetation. Cropping two (or in a few vigorous species even three) times in a season, leaving between each harvest an interval of time sufficient for the development of considerable aerial growth, did not jeopardize the forage yield. MCCARTY (**7**) harvesting in Colorado *Agropyron smithii* and *Bulbilis dactyloides* four times in a season, found that growth and the food reserves were so curtailed that the mortality due to winter killing was very high during the

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period of diplomacy. Removal of the herbage at such intervals as to result in a reduction of the total aerial dry matter produced was invariably reflected in a decreased root development and in a lesser quantity of food stored in the stem bases and in the roots themselves. Poor development of the root system resulted in the production of an increasingly small quantity of herbage in the following season. The earlier work of ELLETT and CARRIER (**3**) on blue grass pastures brought out the conclusion that "the total yield of dry matter varies inversely with the number of times the pasture is cut during the growing season." The results of STAPLEDON (**12**) and of GRABER, NELSON, LEUKEL, and ALBERT (**4**) are similar. They concluded further that the root systems of grasses are very sensitive to the cutting of the aerial parts.

As previously stated, studies of the anatomy of roots of Gramineae are limited, despite the apparent economic application of such investigations to yield and longevity of this important family of plants. The following texts, however, are somewhat pertinent to the study in question: DE BARY (**1**), HABERLANDT (**5**), EAMES and MACDANIELS (**2**), and WEAVER (**13, 14**).

JACKSON (**6**) in the study of barley roots concluded that the root system of a well developed barley plant consists of two forms—a thin branched type, and a thick unbranched type with abundant root hairs. The unbranched root arises from the first node above the grain. Its chief function appears to be that of providing ample water and nutrient salts at the time that vigorous growth is taking place. This function is accomplished by the presence of abundant root hairs and an increased number of large vessels and central ducts, as well as the existence of a stele composed largely of thin-walled elements. Measurements of the cross-sections of the roots, some of which were developed in soil and others in water cultures, were taken in two dimensions at right angles to each other, that of the stele, the ducts, and the entire cross-section of the root. These ratios were obtained by taking as area the product of the two diameters "since the area of an ellipse is proportional to that of its escribed rectangle." The ratio was expressed as $\frac{\text{area stele}}{\text{area ducts}}$. Other ratios of area were those of $\frac{\text{whole root}}{\text{ducts}}$ and $\frac{\text{whole root}}{\text{stele}}$, but these were found to be inconsistent.

Methods of study

THE PLANTS.—Because of the detailed character of the work, only two plant species were used in the experiment. The one was *Stipa pulchra*, a perennial bunch grass which typically occurs in a well developed soil "A" horizon and is recognized as a climax species in the true savanna of the Sonoran life zone of California; the other was *Bromus hordeaceus*, an an-

nual of Mediterranean origin now found extensively in the Sonoran zones. Both species are of great value as pasture plants.

THE CULTURES.—In order to facilitate critical study and measurement of the roots and to eliminate the variables of soil and sand, a water culture, formulated by Professor D. R. HOAGLAND, was used. The culture solution, in addition to the regular constituents, contained in the formula a small quantity of so-called "A-Z" solution, which served to offset any deficiency due to the need of rare elements. The constitution of the stock and culture solutions is shown in table I.

TABLE I

SALTS	MOLAR CONCENTRATION, PER LITER STOCK SOLUTION	MOL SOLUTION PER LITER	IONS
	gm.	*cc.*	*ppm.*
$Ca(NO_3)_2 \cdot 4H_2O$	236.2	3.9	Ca 157
KNO_3	101.1	3.6	NO_3 709 K 181
$MgSO_4 \cdot 7H_2O$	246.5	2.2	Mg 55 SO_4 217
KH_2PO_4	136.2	1.1	PO_4 105

The culture required 2 cubic centimeters of a 0.5 per cent. solution of ferric tartrate per liter of solution, to supply iron.

THE TISSUES.—Root tips were taken from adventitious roots of the two species studied when the plants had been grown for 105 days in the water cultures. During this period of growth one set of plants had been harvested by cutting within one-half inch of the stem base on seven occasions at 15-day intervals; the other set of plants had not been harvested or otherwise treated during the period of growth. The root segments were cut at 2.5 cm. from the growing point.

FIXATION.—Part of the material was fixed with Allen's solution; the other part with alcohol-formalin-acetic no. 1 solution for a period of 70 hours. Allen's solution was prepared as follows:

Chromic acid	1 gram
Acetic acid	1 gram
Urea	0.5 gram
Distilled water	100 cc.

The material was then washed in running water for 12 hours after fixing.

The alcohol-formalin acetic no. 1 solution was prepared as follows:

50 per cent. alcohol	100 cc.
Formalin	6.5 cc.
Acetic acid	2.5 cc.

No washing was necessary following fixing.

EMBEDDING.—Following fixation the material was dehydrated by carrying it through a graduated series of increasing alcoholic concentrations to absolute alcohol. The material was then infiltrated with a paraffin solvent, the alcohol being displaced by benzol. The material was then infiltrated with paraffin by adding paraffin shavings to the benzol which contained the plant material. The vials containing this material were kept in a sparkless paraffin oven for 24 hours. Following this the paraffin-benzol solution was decanted and melted paraffin was added. The paraffin was changed twice during the 24-hour period, following which the material was allowed to remain 48 hours in the oven.

The material was then poured into pasteboard boxes and cooled with waste alcohol. The blocks were sectioned with a rotary microtome in thicknesses of 7 microns and 15 microns, respectively, and were mounted on glass slides in the usual manner. The slides were passed through xylol, alcohol, and water to remove paraffin and to infiltrate the section with the water to facilitate staining. A modification of Haidenhain's iron-alum haematoxylin stain was used.

MEASUREMENTS.—Measurements were made with a Filar micrometer which had been calibrated with a stage micrometer. Two diameters of the cross-section of the whole root, the stele, and the ducts were obtained.

Results and discussion

There was a marked difference in the size of the roots of the plants harvested compared with those not harvested. In *Stipa pulchra* the average diameter of the cross-section of the whole root was 0.827 mm. in the plants not harvested and 0.517 mm. in those harvested seven times at 15-day intervals, or a difference of 37.5 per cent. (table II, figs. 1 and 2). The area of the cross-section of the whole root was 0.684 sq. mm. in the plants not harvested and 0.267 sq. mm. in those harvested, or a difference of 61.0 per cent. The diameter of the stele was 0.367 mm. in the plants not harvested and 0.227 mm. in those harvested, or a difference of 38.2 per cent. The area of the stele was 0.135 sq. mm. in the plants not harvested and 0.052 sq. mm. in those harvested, or a difference of 61.5 per cent. The number of ducts averaged 9 in the untreated plants and 5 in those harvested, a difference of 44.5 per cent. The total area of the ducts was 0.009 sq. mm. in the plants not harvested and 0.004 sq. mm. in those harvested, or a difference of 55.5 per cent.

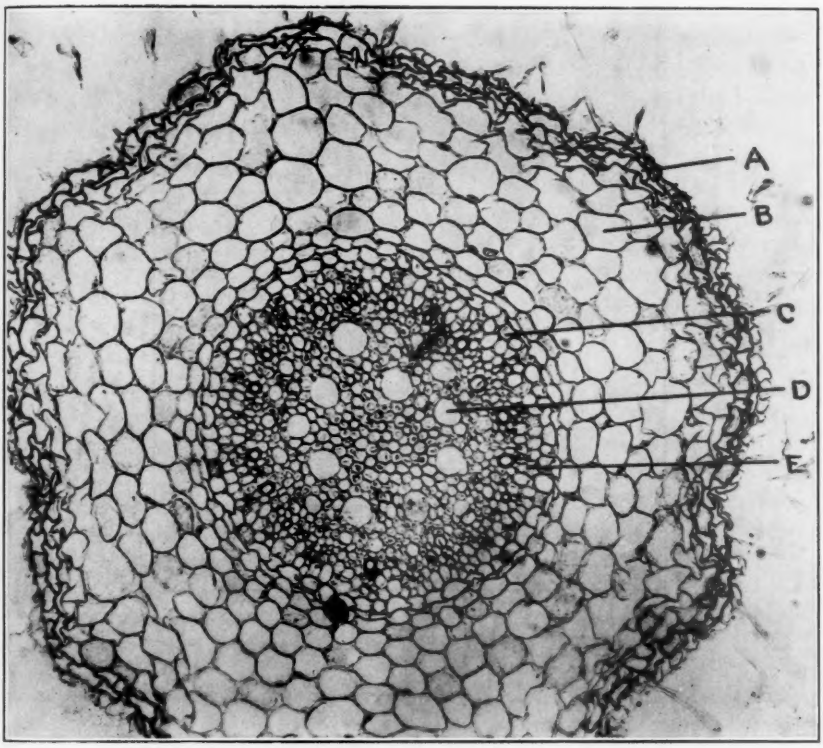

FIG. 1. Cross-section of root of *Stipa pulchra*, not harvested. 143×.

In *Bromus hordeaceus* similar results, although somewhat less marked, were obtained in the roots of harvested and of unharvested plants (table II, figs. 3 and 4). Here again, the harvested plants were clipped at 15-day intervals over a period of 105 days. The dates of cutting were the same as for *Stipa pulchra*.

In the plants of *Bromus* not harvested the diameter of the whole root was 0.606 mm., as compared with 0.434 mm. in those harvested, with a difference of 28.4 per cent. The area of the whole root was 0.367 mm. in the untreated plants as compared with 0.189 mm. in those harvested, a difference of 48.5 per cent. The diameter of the stele was 0.237 mm. in the control plants, as compared with 0.181 mm. in those harvested, with a difference of 23.7 per cent. The area of the stele was 0.056 sq. mm. in the untreated, as compared with 0.033 sq. mm. in the harvested plants, with a

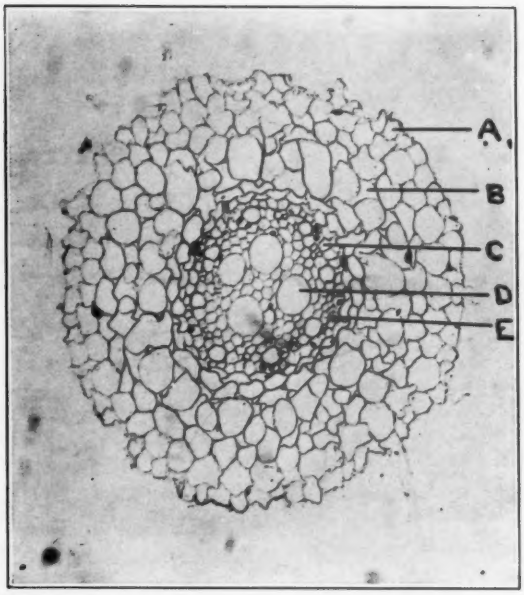

FIG. 2. Cross-section of root of *Stipa pulchra*, harvested frequently. 143 ×
A, epidermis; B, cortex; C, endodermis; D, vessel; E, pericycle.

difference of 41.1 per cent. The number of ducts averaged 6 in the untreated plants, as compared with 2 in the harvested plants, with a difference of 66.6 per cent. The total area of the ducts was 0.008 sq. mm. in the untreated plants, as compared with 0.003 sq. mm. in those harvested, with a difference of 62.5 per cent.

The marked difference in the size of the roots in the *Bromus* plants harvested as compared with those untreated is probably accounted for by (1) the decreased elaborated nutrition of the harvested plants as a whole, (2) the decreased absorptive capacity of the roots, and (3) a corresponding decreased transpiration stream, all of which results from the frequent reduction in leaf area. Regeneration of the aerial growth was strongly stimulated by harvesting the tops. This likewise resulted in an appreciable prolongation of the growth cycle of the herbage. This vigorous regeneration growth accounts for an unequal utilization of the elaborated foods, resulting in weak root growth as a whole and a corresponding decrease in the size and the differentiation of the root cross-section. The presence of a larger number of ducts in the untreated plants would appear to be associated with their relatively larger requirements for water and mineral

TABLE II

COMPARATIVE ANATOMY OF PLANTS HARVESTED FREQUENTLY AND THOSE NOT HARVESTED

	Stipa pulchra CONTROL	*Stipa pulchra* HARVESTED	DIFFERENCE *Stipa pulchra*	*B. hordeaceus* CONTROL	*B. hordeaceus* HARVESTED	DIFFERENCE *B. hordeaceus*
			per cent.			*per cent.*
Diameter whole root in mm.	0.827	0.517	37.5	0.606	0.434	28.4
Area of whole root in sq. mm.	0.684	0.267	61.0	0.367	0.189	48.5
Diameter of stele in mm.	0.367	0.227	38.2	0.237	0.181	23.6
Area of stele in sq. mm.	0.135	0.052	61.5	0.056	0.033	41.1
Number of ducts	9	5	44.5	6	2	66.6
Area of ducts in sq. mm.	0.009	0.004	55.5	0.008	0.003	62.5

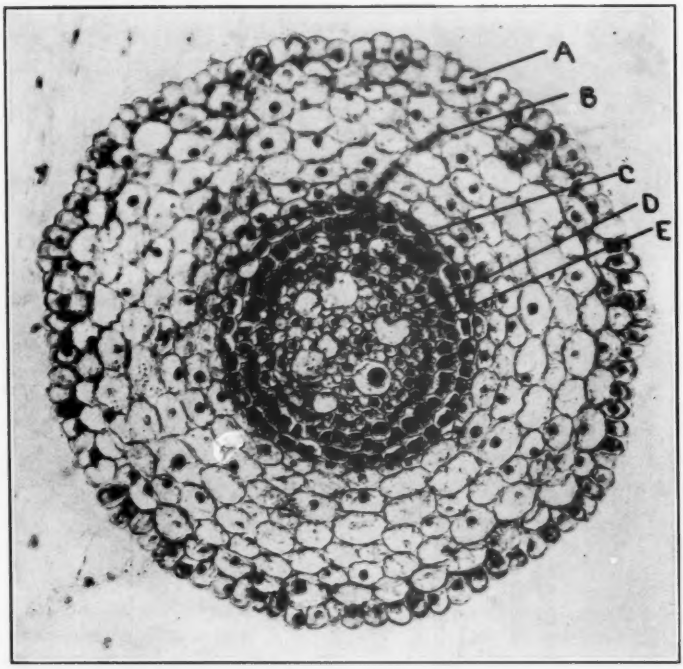

FIG. 3. Cross-section of root of *Bromus hordeaceus*, not harvested. 143×.

salts to compensate for their greater size and the balance in their top-root ratio.

An interesting contrast was noted in the production of root hairs. The roots of *Stipa pulchra* grown in water culture produced root hairs which appeared a short distance behind the meristematic region of the root tip. These organs, however, died off as newer ones were formed nearer the root tip. In contrast, the roots of seedlings of *Bromus hordeaceus* produced hairs, but as the plants became older the roots were devoid of such growth and were quite smooth. Possibly this behavior was due in part to adaptation of the plant to the oxygen supply of the solution; the fact that the more elaborate root system was more exposed to the air by the lowering of the solution in the jars; or because sufficient gas was supplied by the more elaborate aerial parts, as observed by SNOW (7). In neither *Stipa pulchra* nor *Bromus hordeaceus*, however, did the frequently clipped plants, after the first month of growth, produce root hairs.

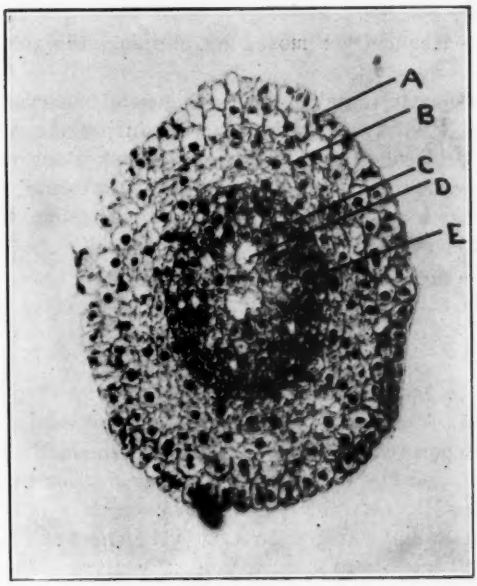

FIG. 4. Cross-section of root of *Bromus hordeaceus*, harvested frequently. 143×. A, epidermis; B, cortex; C, endodermis; D, vessel; E, pericycle.

Conclusions

1. Water cultures as here used have proven of great value in the study of root behavior and anatomical differentiation of the species employed.

2. Frequent removal of the aerial growth in *Stipa pulchra* resulted in a poorly developed root structure, in that the diameter of the whole root, the diameter of the stele, and the number of ducts were invariably smaller than in roots of the same age of untreated plants.

3. Frequent removal of the aerial growth of *Bromus hordeaceus* also resulted in a poorly developed root structure, in that the diameter of the whole root, the diameter of the stele, and the number of ducts were smaller than in roots of the same age of untreated specimens.

4. In the cultures where the leaf blades of *Stipa pulchra* were not harvested, root hairs were produced. In contrast, the roots of unharvested plants of *Bromus hordeaceus*, although producing root hairs when very young were later devoid of such structures. This disappearance of the root hairs might be accounted for by the presumed accommodation of the species to the oxygen supply.

5. Where the succulent aerial growth of *Stipa pulchra* and *Bromus hordeaceus* was frequently removed by clipping, the roots were devoid of hairs.

6. This experiment supports the more general observations of similar leafage removal studies with many species during the period of active growth under field conditions. Where it is important to maintain the herbaceous cover, as for pasturage, or where an elaborate, robust root system is necessary to bind the soil in order to control erosion, frequent leafage removal should be avoided.

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DISTRIBUTION OF MOISTURE, DRY MATTER, AND SUGARS IN THE MATURING CORN STEM[1]

F. A. Welton, V. H. Morris, and A. J. Hartzler[2]

(WITH SIX FIGURES)

Introduction

A series of physiologic studies of corn has been undertaken cooperatively by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. D. A., and the Dept. of Agronomy, Ohio Agricultural Experiment Station. Among the studies inaugurated, one related to the distribution of moisture, dry matter and sugars in the stem during the period from tasseling to maturity. The results obtained in the study are presented in this paper.

Material and methods

The investigation has been carried on for two years. There was used in 1927 the variety Clarage, a yellow dent corn which matures about midseason at Wooster, and in 1928 Burr Leaming, a double cross of four selfed lines of dent corn, late maturing. The corn was grown on a Wooster silt loam which is in a good state of fertility.

The first samples were taken as soon as the plants were fully tasseled and the sampling was continued at weekly intervals to maturity. The samples consisted of 6 plants each having 12 nodes. The stalks were cut off at the surface of the soil. The leaves, including the sheaths, the tassels, and the ears were all discarded. The stems were then divided into segments, the division in each case being made at the growing point just above the node. The term "segment" as used in this paper refers to a section of stem which consists of an internode and the node above. Each stem, therefore, was divided into 12 segments which were numbered consecutively, the lowest being designated always as "1". The corresponding segments of the 6 plants were grouped together to form composite samples representing the different segments. For each date of sampling, therefore, a series of 12 composite samples was obtained.

The segments were cut into small pieces and aliquot samples preserved in boiling alcohol. Later these samples were placed in Soxhlet extractors and extracted for 8 hours with 80 per cent. alcohol. The following determinations were made: total dry matter, reducing sugars, and sucrose. An attempt was made to determine starch, but there was not enough present to give a test by the usual quantitative method.

[1] Contribution from the Department of Agronomy, Ohio Agr. Experiment Station.

[2] Associate, Associate and former Assistant in Agronomy respectively.

The data are expressed on the fresh weight basis. The percentage of "residual dry matter" reported is the total dry matter minus the total sugars. MASON and MASKELL (1) have suggested the use of this method of expression on the basis that since the major fluctuations in dry weight of relatively mature plant tissues are due to variations in carbohydrates, it may be assumed that the remaining fraction of the dry weight will be relatively constant.

Results

COMPOSITION OF THE STEM BY SEGMENTS

The percentage composition of the successive segments of the stem as found in the variety Clarage in 1927 is given in table I and presented

TABLE I

COMPOSITION OF SUCCESSIVE SEGMENTS OF THE CORN STEM
VARIETY—CLARAGE, 1927

SEGMENTS	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	*per cent.*	*per cent.*	*per cent.*
1	11.8	84.6	1.2	2.4
2	11.3	84.7	1.4	2.6
3	10.7	84.5	1.7	3.1
4	11.1	83.5	1.9	3.5
5	11.5	82.8	1.9	3.8
6	12.1	81.7	2.0	4.2
7	12.8	80.7	2.1	4.4
8	13.1	80.2	2.2	4.4
9	12.3	80.6	2.5	4.6
10	13.5	80.7	2.2	3.6
11	12.6	81.5	2.2	3.7
12	14.7	79.5	2.2	3.6

graphically in fig. 1. The data are the averages of the eight series of samples taken from tasseling to maturity.

The percentage of residual dry matter increased from 11.8 per cent. in the lower part of the stem to 14.7 per cent. in the upper part. The percentage of moisture, on the other hand, decreased from 84.6 per cent. in the lower part of the stem to 79.6 per cent. in the upper part. The percentage of reducing sugars and sucrose increased from 1.2 per cent. and 2.4 per cent., respectively, in the lower part of the stem to 2.5 per cent. and 4.6 per cent. in the 9th segment, and then decreased slightly.

The percentage composition of Burr Leaming for 1928 is given in table II and presented graphically in fig. 2. These figures represent the averages

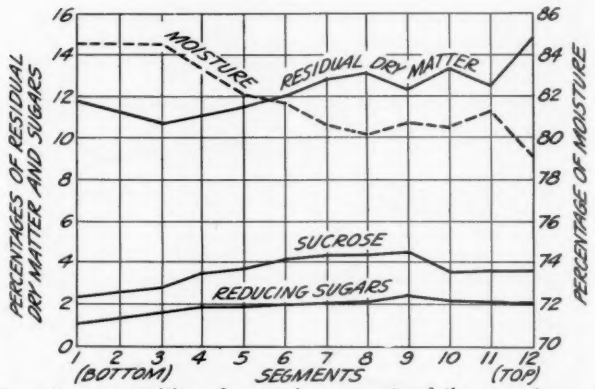

FIG. 1. Percentage composition of successive segments of the corn stem. Average of eight samples taken from tasseling to maturity. Variety—Clarage, 1927.

TABLE II

COMPOSITION OF SUCCESSIVE SEGMENTS OF THE CORN STEM

VARIETY—BURR LEAMING, 1928

SEGMENTS	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	*per cent.*	*per cent.*	*per cent.*
1	11.9	80.5	3.5	4.1
2	12.2	80.4	3.5	3.9
3	13.1	79.1	3.2	4.6
4	13.3	78.6	2.9	5.2
5	14.4	77.4	2.8	5.4
6	14.9	77.0	2.6	5.5
7	16.1	75.9	2.5	5.5
8	16.4	75.4	2.4	5.8
9	16.0	75.8	2.4	5.8
10	16.0	75.7	2.4	5.9
11	15.7	75.9	2.4	6.0
12	17.1	74.6	2.5	5.8

of the six series of samples taken from tasseling to maturity. In general, the composition of the successive segments of Burr Leaming stems showed the same trend as did that of Clarage. The residual dry matter increased from 11.9 per cent. in the lower part of the stem to 17.1 per cent. in the upper part. The moisture content varied from 80.5 per cent. in the lower part of the stem to 74.6 per cent. in the upper part. Reducing sugars de-

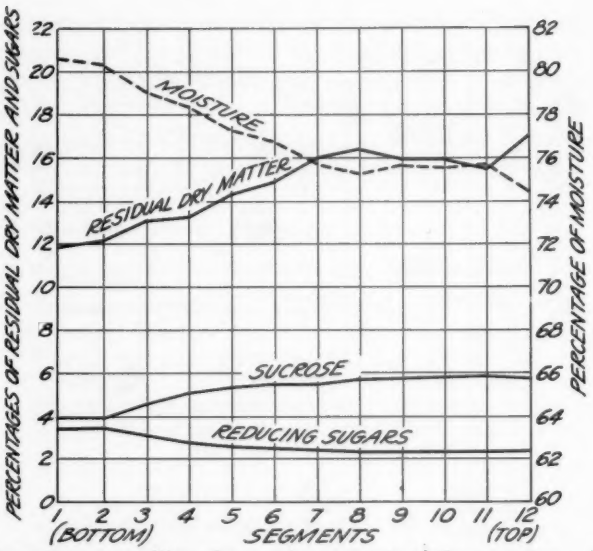

FIG. 2. Percentage composition of successive segments of the corn stem. Average of six samples taken from tasseling to maturity. Variety—Burr Leaming, 1928.

creased from 3.5 per cent. in the lower part of the stem to 2.5 per cent. in the upper, while sucrose increased from 4.1 per cent. in the lower to 5.8 per cent. in the upper part.

SEASONAL CHANGES

The data so far show the gradients of residual dry matter, moisture, and sugars as they occurred between different parts of the corn stem, considering the period between tasseling and maturity as a whole. In order to determine the seasonal changes which took place in the stem, the analyses of the 12 segments for each sampling date were averaged, thus giving the average composition of the stem as a whole for each date.

The percentage composition at different periods from tasseling to maturity for the variety Clarage is given in table III and presented graphically in fig 3. There was a rather rapid increase in residual dry matter from 7.9 per cent. to 12.9 per cent. during the early part of the period, followed by a more gradual increase. There was a correspondingly rapid decrease in moisture content during the early period from 87.1 per cent. to 79.5 per cent. but during the last two weeks the moisture content actually increased, due partly to weather conditions and partly perhaps to

TABLE III

COMPOSITION OF ENTIRE CORN STEM AT DIFFERENT PERIODS FROM TASSELING TO MATURITY
VARIETY—CLARAGE, 1927

DATE	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	*per cent.*	*per cent.*	*per cent.*
August 2	7.9	87.1	2.4	2.6
" 9	11.2	84.4	2.5	1.9
" 16	12.1	82.8	2.5	2.6
" 23	12.9	80.9	1.9	4.3
" 29	13.0	79.5	2.1	5.4
Sept. 7	13.6	79.5	1.5	5.4
" 14	13.7	80.0	1.7	4.6
" 20	14.2	82.5	1.0	2.3

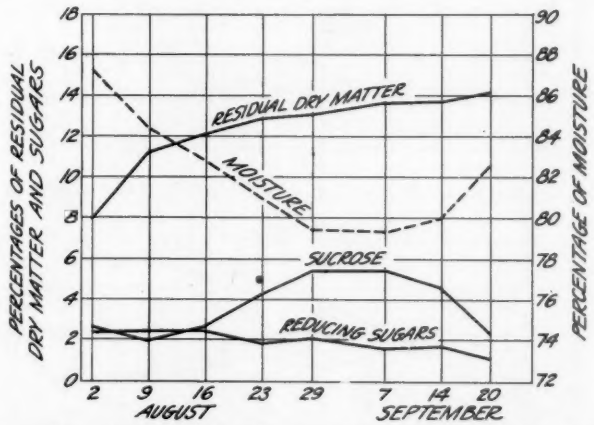

FIG. 3. Percentage composition of entire corn stem at different periods from tasseling to maturity. Variety—Clarage, 1927.

the rapid loss of sugars. The quantity of reducing sugars fluctuated considerably but after the middle of August it trended quite definitely downward. In general the quantity of sucrose increased until the end of the first week in September at which time the ear was well developed, but after that date it declined gradually for a brief time and then more rapidly.

The average composition of the stem of Burr Leaming at successive periods from tasseling to maturity is given in table IV and presented graphically in fig. 4. Residual dry matter increased from 13.9 to 17.4 per

TABLE IV

COMPOSITION OF ENTIRE CORN STEM AT DIFFERENT PERIODS FROM TASSELING TO MATURITY
VARIETY—BURR LEAMING, 1928

DATE	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE
	per cent.	*per cent.*	*per cent.*	*per cent.*
August 10	13.9	80.0	3.1	3.0
" 16	14.0	78.7	3.3	4.0
" 23	14.1	77.9	2.6	5.4
" 30	14.8	76.9	2.7	5.6
Sept. 6	14.5	76.5	2.4	6.6
" 14	17.4	73.0	2.2	7.4

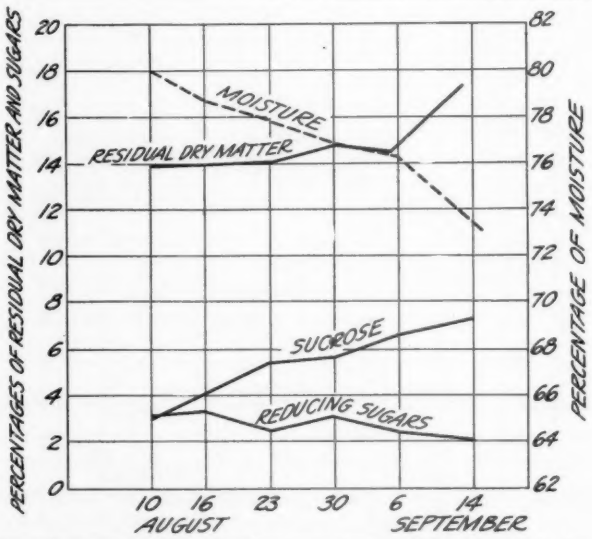

FIG. 4. Percentage composition of entire corn stem at different periods from tasseling to maturity. Variety—Burr Leaming, 1928.

cent. while moisture decreased from 80.0 to 73.0 per cent. On Aug. 16 the reducing sugars were highest, 3.3 per cent. and then they decreased to 2.2 per cent. on Sept. 14; while sucrose increased gradually throughout the period of sampling, making a total gain of 4.4 per cent.

TOTAL AMOUNT PER SEGMENT

Since there is considerable variation in the size of the segments from different parts of the stem as is shown by the green weight and length of

each segment it seemed of interest to calculate the quantity of the various constituents per segment. The results of such calculation for the variety Clarage are given in table V and presented graphically in fig. 5. These

TABLE V

TOTAL QUANTITY OF CONSTITUENTS IN EACH SEGMENT
VARIETY—CLARAGE, 1927

SEGMENT	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE	GREEN WEIGHT OF SEGMENT	LENGTH OF SEGMENT
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*inches*
1	6.8	48.6	0.7	1.4	57.5	4.5
2	10.3	76.7	1.3	2.4	90.7	6.8
3	10.3	82.1	1.6	3.0	97.0	8.1
4	9.7	73.2	1.7	3.1	87.7	8.7
5	8.4	61.0	1.4	2.9	73.7	8.9
6	7.2	48.6	1.2	2.5	59.5	9.2
7	5.8	36.2	1.0	2.0	45.0	8.6
8	4.1	25.6	0.7	1.4	31.8	7.9
9	2.9	19.1	0.5	1.1	23.6	7.9
10	2.3	14.2	0.4	0.6	17.5	7.5
11	1.7	10.6	0.3	0.5	13.1	7.2
12	1.3	8.0	0.2	0.3	9.8	7.1

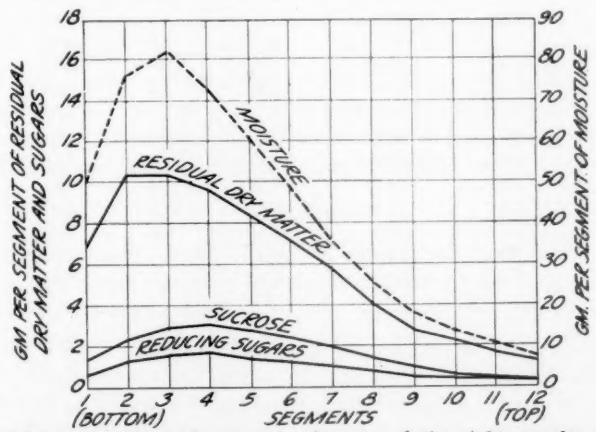

FIG. 5. Total amount in each segment. Average of the eight samples taken from tasseling to maturity. Variety—Clarage, 1927.

data, as mentioned before, represent in each case the average of the series of samples taken from tasseling to maturity.

The total dry matter was a little lower in the first than in the second and third segments, which were equal, but after that it decreased regularly, the twelfth segment containing 9 grams, or 87.4 per cent. less than the second and third, the heaviest two. The moisture content was highest, 82.1 grams, in the third segment—from there it decreased regularly in both directions, reaching a minimum in the twelfth which contained only 8 grams, a decrease of 90.3 per cent.

Residual dry matter decreased from 10.3 grams in the second segment to 1.3 grams in the twelfth. Moisture decreased from 82 grams in the third segment to 8 grams in the twelfth. Reducing sugars and sucrose were highest in the fourth segment, and decreased from 1.7 and 3.1 grams, respectively, to 0.2 and 0.3 gram in the twelfth.

The amounts of the various constituents in each segment of the variety Burr Leaming are given in table VI and presented graphically in fig. 6.

TABLE VI

TOTAL AMOUNT OF CONSTITUENTS IN EACH SEGMENT

VARIETY—BURR LEAMING, 1928

SEGMENT	RESIDUAL DRY MATTER	MOISTURE	FREE REDUCING SUGARS	SUCROSE	GREEN WEIGHT OF SEGMENT	LENGTH OF SEGMENT
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*inches*
1	9.8	62.8	2.7	3.1	78.4	4.8
2	11.4	71.5	3.1	3.5	89.5	6.2
3	11.8	67.6	2.8	4.0	86.2	7.6
4	10.5	58.0	2.2	3.9	74.6	7.9
5	9.2	46.9	1.7	3.3	61.1	8.1
6	7.6	36.8	1.3	2.7	48.4	8.0
7	5.8	26.3	0.9	1.9	34.9	7.3
8	4.1	18.6	0.6	1.4	24.7	7.0
9	3.2	14.3	0.5	1.1	19.1	7.0
10	2.5	11.1	0.4	0.9	14.9	6.8
11	1.9	8.5	0.3	0.7	11.4	6.8
12	1.4	5.8	0.2	0.5	7.9	7.0

The residual dry matter increased from the bottom, reaching a maximum of 11.8 grams in the third segment, from which point it decreased gradually to the top, or twelfth segment, which contained 1.4 grams, or 88.1 per cent. less than the third segment. Beginning at the bottom the moisture content reached a maximum in the second segment from which point it decreased gradually to the top, the twelfth segment containing 91.9 per cent. less than the second. The reducing sugars reached a maximum in the second

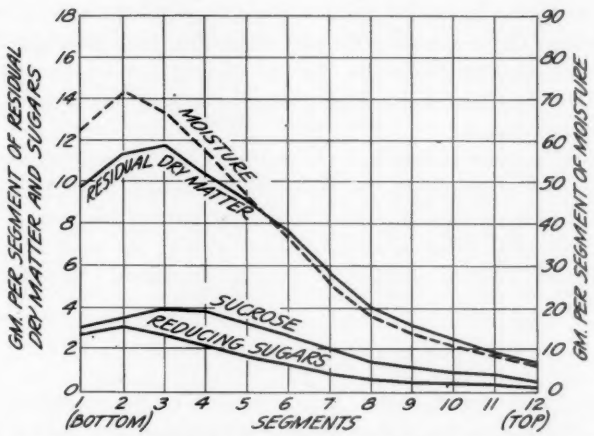

FIG. 6. Total amount in each segment. Average of the six samples taken from tasseling to maturity. Variety—Burr Leaming, 1928.

segment and from there decreased gradually to the twelfth which contained only 0.2 gram, or 93.6 per cent. less than the second. The quantity of sucrose reached a maximum in the third segment from which point it decreased gradually to the twelfth which contained 0.5 gram or only 87.5 per cent. as much as the third or highest.

These results indicate that as far as the relative amounts of residual dry matter, moisture, and sugars are concerned, the maximum was reached in the second, third, or fourth segment and the amounts in the remaining segments decreased rapidly toward the upper part of the plant. Calculation shows that more than 50 per cent. of the total amounts of these constituents in the stem were in the lowest four segments.

Summary

The data obtained in this study of the distribution of residual dry matter, moisture, and sugars in the maturing corn stem indicate the following general conclusions:

The percentages of residual dry matter and sucrose were in general larger in the upper part of the corn stem than in the lower part. The percentage of moisture was larger in the lower part of the stem.

The percentage of residual dry matter increased and the percentage of reducing sugars decreased progressively from the tasseling stage to maturity in both varieties. The percentage of sucrose increased and the percentage of moisture decreased in the Clarage variety in the early part of the season, but the trend of each was reversed at maturity; the per-

centage of sucrose increased and the percentage of moisture decreased in the Burr Leaming throughout the period studied, thus reversing the trend of these two constituents in the Clarage variety in the latter part of the season. This apparent inconsistency may have been due to immaturity of the Burr Leaming.

The total amount of residual dry matter, moisture, and sugars per segment reached a maximum in the region of the second, third, or fourth segment and decreased rapidly toward the upper part of the plant. The lowest four segments, which constitute about 0.25 of the total length of the stem, contained over 50 per cent. of the total amounts of these constituents in the stem.

LITERATURE CITED

1. Mason, T. G., and Maskell, E. J. Studies on the transport of carbohydrates in the cotton plant. Memoirs of the Cotton Research Station. Trinidad. 1928.

EFFECT OF MOLDS ON TEMPERATURE OF STORED GRAIN[1]

JOSEPH C. GILMAN AND D. H. BARRON

(WITH TWO FIGURES)

Although the presence of fungi on stored grain has been known for a long time, the relation of their growth to bin-burning is imperfectly understood. In recent years, however, because of the changes in the methods of handling, much grain has been stored in such condition that molding and bin-burning resulted. Common observation showed that the molding and heating occurred approximately at the same time, indicating a possible interdependence of one upon the other. Grain in elevators, in farmers' granaries, and in transit, all were similarly affected with a correspondingly great loss. The problem, therefore, was a broad one, necessitating the consideration of the relationship between the molds found on bin-burned grain and the production of "heating," the environmental factors favorable to mold growth, and the effects produced.

It is undoubtedly true that temperatures produced in the bin are due to the interaction of several factors. These include the heat of germination itself and temperatures generated by the growth of molds developing on the grain saprophytically. The present investigation was undertaken with the object of isolating and evaluating the rôle of each of these two sources of heat in the temperature increases occurring in stored grains.

The fact that molds may generate high temperatures when grown on suitable organic substances has often been shown in the past. Mention only need be made of the work of COHN (**4**) on barley, MORGENTHALER (**14**) on wheat, rye and speltz, MCHARGUE (**12**) and THOM and LE FEVRE (**19**) on corn meal, KÖNIG (**10**) with cotton, and MIEHE (**13**) on hay to indicate the numerous workers and the wide range of materials on which this phenomenon has been observed. However, there seems to have been some idea that living seeds would not serve as a suitable medium for mold growth.

[1] The investigation reported here was carried on by the Department of Botany in cooperation with the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station as a part of Project 30, "Studies upon the physiology of seed germination." It is published with permission of the Director of the Station.

Since the manuscript was submitted, two very significant papers in the field of thermogenesis by fungi have appeared: MIEHE, H., Die Wärmebildung von Reinkulturen im Hinblick auf die Aetiologie der Selbsterhitzung pflanzlicher Stoffe. Archiv Mikrobiol. **1**: 78–118. 1930; and NORMAN, A. G. The biological decomposition of plant materials. Part III. Physiological studies on some cellulose-decomposing fungi. Ann. Appl. Biol. **17**: 575–613. 1930. Their findings are in accord with those reported here.

Thus, BAILEY (3) and his coworkers as well as earlier investigators, QUAM (17), DUVEL (8) and BABCOCK (2), who were studying the effect of moisture content of seeds upon respiration, seem to have overlooked the fact that unless special care was taken, the same point at which mold growth began was also the point at which they observed marked increases in CO_2 production. PEIRCE (16) and his associates (7), on the other hand, in their studies on the amount of heat liberated by germinating seeds, pointed out that there was no marked rise in temperature in their flasks except where microorganisms were present. JAMES, RETTGER and THOM (9) also, showed that molds as well as bacteria could produce thermogenesis in grain. In one experiment with sterilized corn they produced a temperature of 51° C. with *Aspergillus fumigatus* Fres.

The problem thus became a study of the cause of thermogenesis in the case of oats, barley and wheat by making a comparison of the temperatures caused by the respiration of the germinating grain and those due to fungous growth.

Materials and methods

The seeds used were produced from the Agronomy farm at Iowa State College and with the exception of the barley were all of the crop of the current year. Because of an epidemic of barley scab during that season, seeds from the previous year which were free from scab were substituted as more desirable.

The seeds were divided into 200-gram samples placed in cheese-cloth bags for convenience in handling during sterilization. The bags were removed when the sample was placed in the Dewar flask for observation of temperature rise. In observing the temperature of germination the seeds were sterilized either by submerging them in an alcohol solution of mercuric chloride or by dry heat. The mercuric chloride solution was made up according to the formula used by NABOKICH (15): 15 grams of mercuric chloride, 500 grams of 95 per cent. ethyl alcohol and 3,500 grams of water. After mixing, the solution was allowed to stand over night before being used. The samples were immersed in this solution for 15 minutes and then washed in six changes of sterile distilled water by decantation. Following this treatment the seeds were placed in sterile water for twelve hours before being transferred to the flasks. The dry heat sterilization was carried out according to the method described by ATANASOFF and JOHNSON (1). The grain was thoroughly dried and then held at a temperature of 100° C. for thirty hours. The dry heat method was successful with wheat and oats, but the barley was killed by the treatment and, therefore, that phase of the problem was omitted. After cooling, the seed was covered with sterile water for twenty-four hours and then transferred to sterile Dewar flasks under aseptic conditions.

The chambers used to measure the temperatures of germination and mold growth were commercial thermos bottles, plugged with sterile cotton. Thermometers were inserted through the cotton to record the temperatures. Bottles and thermometers were sterilized with the alcoholic mercuric chloride solution and rinsed in sterile water before using. Readings were made at twelve-hour intervals as long as the temperature continued to rise.

Experimental data

Temperatures of germination

In the experiments with the sterilized grain, a slight rise in temperature occurred in all cases. The summarized results are presented in table I

TABLE I

Temperatures produced in 200-gram samples of sterilized germinating grain

Time	Samples sterilized by dry heat at 100° C. for 30 hours		Samples sterilized by immersion in alcoholic mercuric chloride solution for 15 minutes		
	Oats[1]	Wheat[1]	Oats[1]	Wheat[1]	Barley[2]
days	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*
0	17.0	17.0	17	16.9	17.3
	18.0	16.8	17.8	16.8	17.5
1	18.8	17.0	18.2	17.6	17.8
	21.0	17.0	19.4	18.0	18.6
2	22.8	17.4	20.2	18.2	19.0
	23.8	17.6	20.8	19.6	19.6
3	23.6	18.2	21.4	20.0	20.4
	22.2	18.4	21.2	20.0	20.8
4	21.2	19.0	19.8	20.2	20.9
		19.6		20.2	20.7
		19.8		19.4	
		19.8		19.0	

[1] Composite of five samples.

[2] Composite of ten samples.

and figure 1. With the oats and wheat the data presented are a composite of five samples, while with the barley ten samples were used. The rise in temperature with sterile oats was from 17° C. to 23.8° C. on the third day after heat sterilization, and from 17° C. to 21.2° C. on the fourth day after chemical treatment. That is, the germination of oats raised the temperature of a 200-gram sample from 4.2° C. to 6.8° C. In the case of wheat, after heat sterilization the temperature rose from 17° C. to 19.8° C. in four days; after chemical sterilization from 16.9° C. to 20.2° C. in four days.

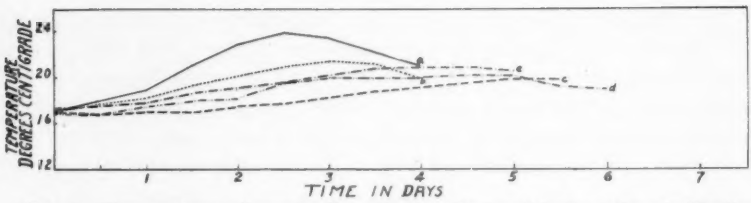

FIG. 1. Temperatures of germination of oats, wheat and barley after sterilization. a—oats sterilized by dry heat. b—oats sterilized by chemical means. c—wheat sterilized by dry heat. d—wheat sterilized by chemical means. e—barley sterilized by chemical means.

The germination of the wheat raised the temperature of a 200-gram sample from 2.8° C. to 3.3° C. With the barley the temperature of germination after chemical sterilization caused a rise of 3.6° C. for a 200-gram sample or a rise from 17.3° C. to 20.9° C. in four days. These data indicate that the high temperatures found in heating grain could not be caused by germination alone, since in no case did the temperature rise above 25° C.—a temperature which was found in a single case among the trials with oats.

Temperatures due to fungous growth

In order to select proper fungi for heat production in grain, a preliminary survey was made of the species of molds present on "heating" grain and three of those commonly found in such materials were selected. They were: *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Brefeld, and *Aspergillus fumigatus* Fresenius. These three species had been isolated in pure culture and grown on corn-meal agar slants. From these slants, subcultures were made to corn-meal in Erlenmeyer flasks and from these cultures the spores were gathered for inoculating the grain in the thermal chambers. Thus all medium was eliminated from the chamber. Wheat, oats and barley were used in the trials. The seeds were sterilized by dry heat in 200-gram samples as in the case of those studied for temperatures of germination. After sterilization the seeds were allowed to cool to room-temperature and then a sample of the grain was removed from the container and its moisture content determined by the Brown-Duvel method. A germination test was made on a second portion and all samples testing less than 90 per cent. germination were discarded. Those samples of proper germinability were then brought to 18 per cent. moisture content by the addition of sterile water and placed in the ice box for 24 hours to allow the added water to diffuse throughout the mass. After this time, the samples were transferred to sterile Dewar flasks prepared in the same way as those used for determining the heat of germination. Spores from the cultures

TABLE II

TEMPERATURES PRODUCED IN 200-GRAM SAMPLES OF STERILIZED GRAIN WITH A MOISTURE CONTENT OF 18 AND 20 PER CENT. BY THREE SPECIES OF *Aspergillus*

TIME	OATS				WHEAT						BARLEY		
					Aspergillus niger		*Aspergillus flavus*						
	Aspergillus niger AT 18 % MOISTURE	*Aspergillus flavus* AT 18% MOISTURE	*Aspergillus fumigatus* AT 18% MOISTURE	CONTROL STERILE GRAIN	AT 18% MOISTURE	AT 20% MOISTURE	AT 18% MOISTURE	AT 20% MOISTURE	*Aspergillus fumigatus* AT 18% MOISTURE	CONTROL STERILE GRAIN	*Aspergillus niger* AT 18 % MOISTURE	*Aspergillus flavus* AT 18% MOISTURE	CONTROL STERILE GRAIN
days	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*	° *C.*
	25.9	25.1	26.8	26	25.7	28.1	28.1	29.7	26.0	25	28.1	33.4	29
	28.5	26.2	28.0	26	29.0	33.4	29.8	30.7	27.2	25	31.1	34.2	27
1	31.7	26.8	29.1	25	32.0	37.1	30.0	30.6	27.5	24	33.6	38.2	28
	36.9	29.2	30.0	25	34.2	41.4	30.4	30.4	28.2	22	38.4	37.4	27
2	42.4	35.5	31.5	24	35.0	45.6	32.5	30.8	28.2	22	41.9	37.7	28
	46.6	41.2	32.7	24	35.5	49.4	34.0	34.1	27.7	23	44.3	41.4	27
3	48.3	47.0	36.5	24	34.0	51.1	35.5	35.8	28.5	23	44.6	43.2	29
	51.2	50.6	40.4	24	31.7	51.2	35.2	38.5	29.2	22	43.6	47.0	27
4	52.2	51.1	45.9	25		51.5		—	29.0	21	44.8	46.2	27.5
	50.8	50.8	49.5	25		51.2		44.8	30.5	21	46.6	47.7	26
5	49.0	47.4	51.0	25		51.2		43.8	31.2		47.3	48.1	26
	49.0		52.1	24		50.5		45.4			—	49.3	—
6			52.6	24				45.5			48.4	49.4	24
			53.2	25							49.2		27.5
7											49.2		24
											50.0		27

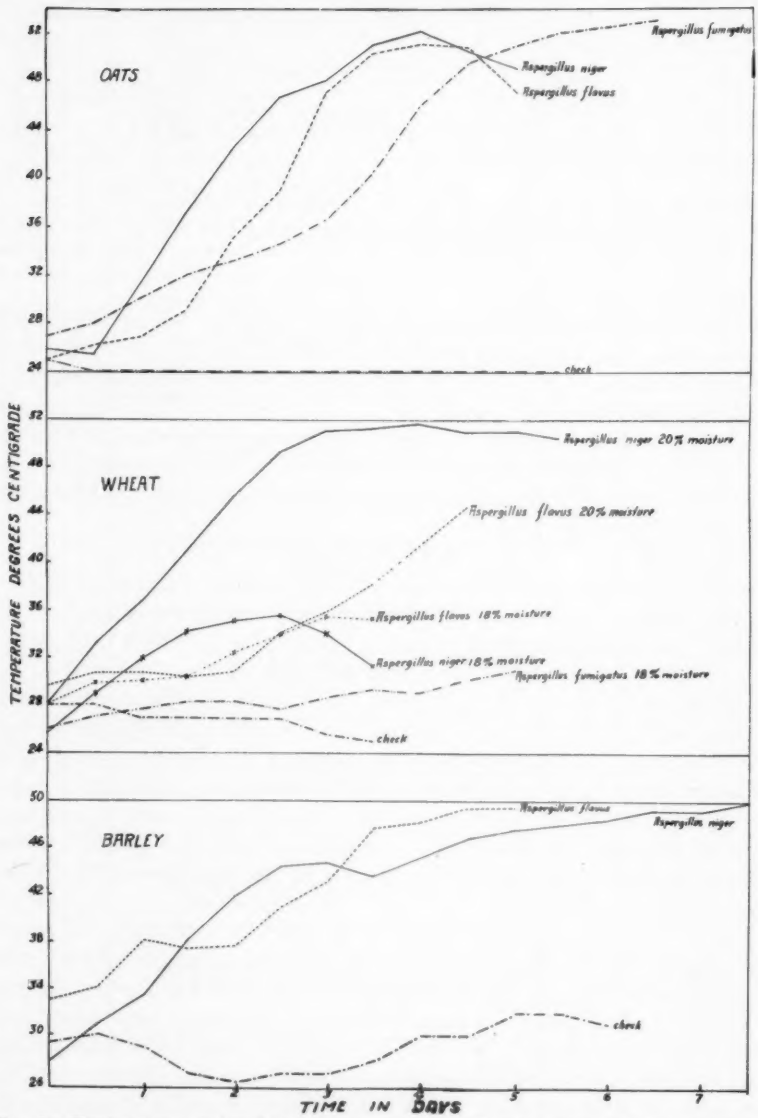

FIG. 2. Temperatures induced in 200-gram samples of oats, wheat and barley by the growth of *Aspergillus niger*, *A. flavus* and *A. fumigatus* at 18 and 20 per cent. moisture content.

were introduced into the flasks at this time, being placed as near the center of the flask as possible by estimation. The introduction of the spores was made in a closed inoculation chamber. Temperatures were read and recorded as in the study of the heat of germination.

The data from the mold studies showed that these fungi were very active in producing heat when grown on moist grain. The summarized data are presented in table II and fig. 2. *Aspergillus niger* raised the temperature in the samples of the three grains at 18 per cent. moisture content used, as follows: oats, 25.9° C. to 52.2° C. or a total of 26.3 degrees; wheat, from 25.7° C. to 35.5° C. or a total of 9.8 degrees; and barley, from 28.1° C. to 50° C. or a total of 21.9 degrees. *Aspergillus flavus* raised the oats sample from 25.1° C. to 51.1° C. or a total of 26 degrees; wheat from 28.1° C. to 35.5° C. or 7.4 degrees; and barley from 33.4° C. to 49.4° C. or 16 degrees. *Aspergillus fumigatus* was used only with oats and wheat, and although it started somewhat slowly the rise in temperature was correspondingly large; oats, from 26.8° C. to 53.2° C. or 26.4 degrees; and wheat, from 26.0° C. to 31.2° C. or 5.2 degrees. In all cases the control flasks of sterile grain showed no appreciable rise in temperature during the experiments. In the case of wheat, two trials of five samples each were made using *Aspergillus niger* and *Aspergillus flavus* at 20 per cent. moisture content. The resulting increases in temperature with *A. niger* from 28.1° C. to 51.5° C. (23.4°) and *A. flavus* from 29.7° C. to 45.5° C. (15.8°) indicate that the amount of heat generated by the molds will vary with the percentage of water in the grain. Presumably there is a minimum, optimum and maximum which is determinable. Furthermore, the different molds differ in the time of their reaction. *A. niger* and *A. flavus* both act rather rapidly bringing the temperature to a maximum in approximately four days, while *A. fumigatus* did not reach its maximum heat production until after the fifth day.

That other fungi in addition to species of *Aspergillus* will produce heat in moist grain, is indicated from a trial made with a *Mucor* (sp. undet.) on oats at 18 per cent. moisture. In this trial four samples were run and the temperature was raised from 19° C. to 44° C. (25° C.) in six days.

One other significant fact may be deduced from these data; that is, the glumed grains, oats and barley, presented a better substrate for the growth of the fungi concerned than did the naked berry, wheat. This observation is borne out by the fact that in storage the glumed grains are considered to be more liable to heating than is wheat.

Summary

In order to study the effect of molds on the temperature of stored grain, three of those most frequently found in moldy grain, *Aspergillus niger* Van

Tieghem, *Aspergillus flavus* Bref., and *Aspergillus fumigatus* Fres., were grown in pure culture on artificial media and inoculated into samples of oats, barley, and wheat, which had been previously sterilized and brought to a moisture content of 18 per cent. The sterilized grain was placed in sterile, pint thermos bottles and the temperatures were obtained by means of thermometers inserted through sterile cotton plugs. Readings were made twice each day. The grain was sterilized by means of heat; 200-gram samples were held at 100° C. for 30 hours. Control experiments, consisting of samples of each grain used without the mold were placed at 18 per cent. moisture in separate flasks, and samples of sterilized grain with sufficient moisture to bring about germination, were carried out in all trials. In addition, samples of sterilized wheat at 20 per cent. moisture content inoculated with *A. niger* and *A. flavus* were included.

The data gathered showed that under the conditions of the trials, the rises in temperature due to germination of oats, wheat, and barley, were 6.8°, 2.6°, and 3.6° C., respectively. *A. flavus* raised the temperatures of oats, wheat, and barley at 18 per cent. moisture content, 26°, 7.4°, and 16° C., respectively. *A. niger* raised the temperatures of these grains 26.3°, 9.8°, and 21.9° C., respectively. *A. fumigatus* raised the temperature of oats at 18 per cent. moisture, 26.4° C. and wheat 5.2° C. At 20 per cent. moisture content, *A. flavus* and *A. niger* raised the temperature of wheat, 15.8°, and 23.4°, respectively. These facts strongly suggest the probability that in bins of stored grains marked increase in temperature may be ascribed to mold growth.

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CALLUSING OF COTTON STEM CUTTINGS

H. E. Rea

(WITH PLATE)

In a previous paper (**6**) the ability of cotton stem cuttings to callus and root was reported. In this earlier experiment rather meager results were obtained. Only 43 per cent. of the cuttings used callused, while barely 6 per cent. rooted. A number of trials have subsequently been made without materially increasing the percentages of cuttings rooted.

The stem cuttings of some plants do not necessarily callus before forming roots but, in general, callusing is associated with the rooting of stem cuttings (**4, 7**). All of the stem cuttings of cotton that have been observed to root have first callused so that the formation of calluses may probably be considered an essential accompaniment to the rooting of cotton cuttings. Thus little hope is entertained for obtaining a very high percentage of rooting before a higher success at callusing cotton stem cuttings is secured. With much the same idea that germination tests are conducted with seed, it was decided to make a number of callusing trials with cotton stem cuttings. While the securing of callus does not insure the rooting of a cutting ordinarily, the factors that favor callusing also favor rooting (**4, 7**). From such callusing experiments it was hoped that a better idea of the optimum conditions for rooting cotton stem cuttings might be more rapidly determined.

Early in the fall of 1929 some Osage-orange (*Toxylon pomiferum*) stems enclosed within an ordinary glass fruit jar, which had been partially filled with moist soil before closing, was observed to have callused very profusely. This was not considered unusual for Osage-orange stems but it did suggest the use of this type of jar as an interesting propagating chamber. When glass jars are loosely closed with the screw top, they provide most of the features of the Wardian propagating case (**3**) in a unit very convenient for experimental work.

Accordingly, on November 13, 1929, a number of callusing trials, using the glass jar as a propagator, was set up. The process of preparing the material and setting up the experiment consumed the three days prior to the thirteenth, but all of the propagators were not finally in place until that day. From previous experience it was known that handling the cuttings during the process of callusing was injurious so that few observations were made prior to the completion of the test. Previous experience also indicated that approximately four weeks were required for the cuttings to callus so that it was decided to allow six weeks for the completion of the

experiment. On December 22 and 24 all propagators were opened and records made of the number of cuttings with clean basal callus within each propagator chamber.

A certain procedure was established as standard on the basis of usual recommendations (**1, 3, 9**). These standard practices were used with the bulk of the cuttings handled, while variations from these standards were made from group to group. These standards and variations made are described below.

Plant materials

Cutting wood

In collecting the cotton plants from the field all the leaves and lateral branches were trimmed from the central stalk. Only the main stem was used in making cuttings. As a usual procedure the main stem was divided roughly into three sections: the upper one-third furnishing the soft wood cuttings; the middle one-third the medium mature cuttings; and the lower one-third the mature cuttings. In the case of the soft wood cuttings this division was not always strictly held to, as portions of the upper one-third were sometimes too mature to be classed as soft wood. In no case was any wood used that was so tender as to wilt down under the conditions of the experiment.

All cutting wood was used immediately upon collecting it from the field except in one lot where the storage of the wood for twelve hours was tested. No special treatment was given the wood under storage as it was held at dry air condition in the laboratory. Fresh cuts were made on the wood stored as the cuttings were placed in the propagators.

Standard cuttings.—The standard cutting used throughout the majority of the tests consisted of stems from 10 to 15 centimeters in length containing from 3 to 4 nodes. Both the apical and the basal cuts were made with a sharp knife and the basal cut was made closer to the node than the apical cut. All standard cuttings were cleaned of any buds, bracts and leaves which were not removed in the field. All cuts made with the knife were sloping or angular. In specific experiments variations from the standard cuttings were made to test the particular variation.

Two node cutting.—The short cuttings were from 5 to 7 centimeters in length and included a single internode with 2 nodes. The cuts were made as with the standard cutting.

Rough cuts.—Cuttings made of both standard and 2 node size, where the cuts were made with sharp pruning shears and only the leaves, buds, and bracts removed in the field trimming, were classed as rough cuts. All cuts made with the shears were straight or square.

Embedding of cuttings

In using standard length cuttings placed in soil, the base of the cutting was usually covered to a depth of about 5 centimeters. In one series the amount of soil itself was varied. In these experiments the cuttings were covered from 4 to 8 centimeters in accordance with the volume of soil used. When *Sphagnum* moss was used the butts of the cuttings were covered less than 4 centimeters. In the case where no solid medium was added the cuttings rested on the bottom of the propagator.

In a number of propagators the bases of standard cuttings were inverted and left exposed in the moist air above the soil material used. The tops of the cuttings in these jars were embedded in the soil about 5 centimeters. Inverted cuttings were not used with media other than soil materials.

The two node cuttings were too short to permit covering the bases sufficiently to hold them erect so that they were allowed to lie flat on the surface of the soil material, leaving the entire cutting exposed. With other propagators in which two-node cuttings were used the cuttings were completely covered with the soil material.

Potassium permanganate treatment

Curtis (2) was able to hasten the rooting of *Ligustrum ovalifolium* by various treatments of potassium permanganate. To test the possible value of this chemical in stimulating callusing in cotton one hundred and forty cuttings were treated for thirty minutes. The basal cuts of four separate lots of thirty-five cuttings each were submerged in 2, 1, 0.5, and 0.25 per cent. solution of potassium permanganate in water for thirty minutes and then washed with water before placing them in the propagators. As a check against the potassium permanganate treated cuttings one lot of thirty-five cuttings was allowed to stand in tap water for the same length of time, under similar conditions. In each propagator in these tests five cuttings of untreated *Ligustrum japonicum* were also included as a check against the general condition for callusing within the propagator.

All the cuttings in these experiments were placed in ordinary quart fruit jars along with such other materials as are described under media. The jars were closed by screw tops without any rubbers. No attempt was made to modify the light condition under which propagators were placed. The fruit jar gives in a small unit propagation cases very similar to the Wardian propagating case when the top is loosely screwed on. The normal number of cuttings placed within each propagator was 10. To determine results of crowding the cuttings, twenty cuttings per propagation were used in a number of cases.

Media

Soil material

Fine gravel.—The fine gravel material used contained rounded quartz pebbles ranging in size from slightly more than one fourth inch in diameter to slightly more than one fourteenth of an inch. The screening of the fine gravel through various sized screens showed that 22 per cent. by weight failed to pass through the screen with 4 meshes to the inch; 63 per cent. was held back by a screen with 6 meshes, and 15 per cent. by a 14-mesh screen. All of the material passed readily through a 2-mesh screen while none passed through the 14-mesh screen. The moisture content of this material at the beginning of the experiments was 2.7 per cent. and no water was added during the course of any of the trials.

Coarse sand.—The coarse sand consisted of much finer quartz particles most of which were angular, although a few of the largest particles were rounded. A screen with 6 meshes retained 11 per cent. by weight of the larger particles; while 54 per cent. passed through a screen of 12 meshes but were retained by the 14-mesh screen. All of the material passing through the 14-mesh screen was retained by the 20-mesh screen, and made up 35 per cent. of the total weight of the composite samples of coarse sand. The coarse sand continued 4.6 per cent. moisture.

Fine sand.—The fine sand contained particles which passed through the 12-mesh screen but failed to pass through the 20-mesh to the extent of 10 per cent. by weight. The material that passed the 20-mesh screen but failed to pass through the 40-mesh screen made up 85 per cent. of the samples while 5 per cent. passed through the 40-mesh screen but was retained by the 60-mesh screen. This material contained 8.2 per cent. moisture.

Houston black clay.—This material was surface soil of typical Houston Black Clay and contained 28.2 per cent. moisture. In this condition it was very friable.

Volume of soil material.—In sections of the experiment the volume of soil was varied. The jars were filled at the rates of 200, 350, and 500 milliliters of soil in the various sections of the test. The total value of the jars was found to be 965 milliliters so that 765, 615, and 465 milliliters of the space in the jars above the respective volumes of soil were occupied by air.

Soil moisture.—The moisture content of each material used was determined at the beginning of the experiment and is given under the description of the specific material. Unfortunately, determinations of the final moisture of the soil at the end of the experiment were not made. However, the phenological studies of the cuttings indicated that little or no moisture

was lost from the propagation chamber during the test period. In order to furnish some evidence to support these observations a separate set up was tested later. On February 20 moisture determinations were made on four separate lots of Houston black clay and one lot of coarse sand. These lots were divided into four separate fractions and treated as shown in table I for six weeks and the determination of moisture made at the end of that time.

TABLE I

AVERAGE PER CENT. OF MOISTURE IN HOUSTON BLACK CLAY AND COARSE SAND AFTER STORAGE IN LOOSELY CLOSED GLASS JARS FROM FEBRUARY 29–APRIL 4, 1930

LOCATION FROM FEB. 20–APR. 4	HOUSTON BLACK CLAY		COARSE SAND
	JARS FILLED 3/4 FULL	JARS FILLED 1/4 FULL	JARS FILLED 3/4 FULL
INITIAL PER CENT.	37.7*	37.7	3.8
WEATHER PLAT 9″ UNDERGROUND	38.3	.....	.....
ROOM NO. 1	37.6	37.6	3.7
GREENHOUSE	38.7	.....	.....

* The average percentage of moisture recorded in the table is the result of four separate determinations.

SPHAGNUM MOSS

The Sphagnum moss used was the common commercial grade, unsterilized. The moss was thoroughly saturated and then squeezed as dry as possible by hand.

In several series no medium other than the air contained within the jar was used. In one series both the cuttings and jar were washed with tap water and immediately drained. In this series such water as clung to the jar and cuttings was left in the jar. In a second series the unwashed cuttings were placed in jars that had been previously washed in tap water. In a third set up both the cuttings and jar were left without introducing any free water.

Temperature

Although the temperature could not be controlled directly for the benefit of this experiment, four locations were available where daily temperature records were being kept. In table II is given the weekly mean and also the maximum and minimum temperatures for each location throughout the course of the experiment.

TABLE II

AVERAGE AND EXTREME TEMPERATURES PREVAILING THROUGHOUT THE EXPERIMENT

DATE WEEK ENDING	AVERAGING TEMPERATURE			
	WEATHER PLAT 9″ UNDERGROUND	ROOM NO. 1	ROOM NO. 2	GREENHOUSE
	° C.	° C.	° C.	° C.
Nov. 16	16.0	16.0	14.0	16.0
Nov. 23	12.0	15.0	17.0	15.0
Nov. 30	9.0	14.0	16.0	21.0
Dec. 1	9.0	15.0	17.0	21.0
Dec. 14	9.0	21.0	21.0	27.0
Dec. 21	14.0	17.0	18.0	21.0
Average	11.5	16.3	17.3	20.2
Maximum	18.0	28.0	28.0	36.0
Minimum	4.0	3.0	3.0	4.0

Results

The percentage of cuttings callusing under the various conditions just outlined was generally low; but in a few cases the results were excellent. The data concerning the percentage of callusing in the various trials are given in table III.

Selection of the proper wood is considered very important in securing a high percentage of callusing with any kind of cuttings (**5, 8**). Some types of wood apparently will callus under a wide variety of conditions while other wood will callus only feebly under the most careful and specialized environment. Cotton stem cuttings appear to respond only to a narrow range of conditions. In previous trials soft wood of cotton has been callused in an environment under which mature wood has shown little tendency to callus. Yet under the environment provided in this recent experiment the reverse was true. Soft wood cuttings were callused in the propagators in only 6.1 per cent. of the trials while the propagation callusing was increased as more mature wood was used. Out of a large number of trials made 25.9 per cent. of the medium mature, and 46.8 per cent. of the mature cuttings produced calluses.

Immediate use of the cutting wood after it has been taken from the plant is usually recommended as conducive to the best results (**1, 3, 9**). The bulk of the cutting wood collected for these trials was used immediately after collecting but a small portion was stored for twelve hours prior to making the cuttings. The records in table III show that 31.7 per cent.

TABLE III

PERCENTAGE OF CALLUSING UNDER THE CONDITIONS TESTED

CONDITIONS OF EXPERIMENTS		TOTAL NO. CUTTINGS	CUTTINGS CALLUSED	
			NUMBER	PER CENT.
Cutting wood Condition Degree of maturity	soft	196	12	6.1
	medium	548	142	25.9
	mature	474	222	46.8
Freshness	12 hrs. storage	110	64	58.2
	immediate use	1204	382	31.7
Type of cut	rough	130	43	33.1
	smooth	1174	403	34.3
Position of cutting	upright, base covered	486	132	27.2
	inverted, base exposed	80	79	98.7
	horizontal, completey covered	40	21	52.5
	horizontal, completely exposed	263	160	60.8
Chemical treatment	water (check)	35	24	68.6
	2 per cent. $K_2MN_2O_8$	35	5	14.3
	1 per cent. $K_2MN_2O_8$	35	6	17.1
	0.5 per cent $K_2MN_2O_8$	35	11	31.4
	0.25 per cent $K_2MN_2O_8$	35	9	25.7
	Ligustrum (check)	175	168	96.0
Condition in propagating case	crowded, 20 cuttings each	400	194	48.5
	non-crowded, 10 cuttings each	490	185	37.8
Media for callusing	fine gravel	683	245	35.9
	coarse sand	115	52	45.2
	fine sand	146	23	15.9
	Houston black clay	115	66	57.4
Depth of soil	2 inches deep	823	289	35.1
	3 inches deep	161	70	43.5
	4 inches deep	40	5	12.5
Other media air in	Spaghnum moss	25	0	0
	jar and cuttings H_2O wash	60	5	8.3
	cuttings H_2O wash	40	5	12.5
	jar and cuttings dry	142	27	19.0
Temperature	buried underground 11.5° C.	180	0	0
	room No. 1 16.3° C.	430	79	18.4
	room No. 2 17.3° C.	380	112	29.5
	greenhouse 20.2° C.	220	82	37.3

and 58.2 per cent., respectively, of the immediately used, and the stored cuttings produced callus tissue. The actual percentage was in favor of storage. It is possible that the storage period improved the cutting wood. It is at least evident that delaying the use of the wood for twelve hours did not injure it.

A clean, smooth, sloping cut in preparing the material for propagation is favored in ordinary procedure (**4**). Such cuts were standard for most of the cutting wood handled; however, one lot of wood was crudely prepared with pruning shears. From the results obtained as presented in table III, it will be seen that about equal percentages of callusing were secured in the two groups. In the lots where cuts were carefully made with a sharp knife 34.3 per cent. of the cuttings callused, while in those prepared with less care with shears, 33.1 per cent. callused.

The placing of the cuttings in the medium is often manipulated to provide optimum conditions for callusing. In some instances hardwood cuttings are buried in moist sand beds with the base of the cuttings up (**1, 3**). This practice is especially common where sunlight is the only source of heat and the upper layer of the sand is warmer. Ordinarily, however, the cuttings are placed upright with the bases covered sufficiently with the medium to hold them erect and to prevent drying. Such procedure was the standard method of placing cuttings in this test. Several variations were also studied. In one case, the standard placement was completely reversed. The tops were buried in the medium and the base of the cuttings were left protruding in the air contained within the propagator. Several lots of short cuttings were used and as they could not conveniently be placed vertically they were allowed to take a horizontal position. One group of shorter cuttings was left exposed on the surface of the medium while another was completely covered. From a study of the section of table III, presenting the records of these variations in treatment, it is seen that 98.7 per cent. of the inverted long cuttings callused as compared with only 27.2 per cent. of those not inverted. Both groups of the shorter cuttings callused fairly well, as 60.8 per cent. of those completely exposed, and 52.5 per cent. of the cuttings completely covered callused. It should be noted that exposure to moist air in both cases gave the highest results. Wherever upright placement was followed, a great many of the exposed tops callused; however, such calluses are not considered in the present analysis. In the group of short cuttings callusing on both ends was general. There is little doubt that the moist air confined within the propagators above the soil material presented the optimum conditions for callusing.

Chemical treatment of the base of certain cuttings has stimulated callusing. By treating cuttings of *Ligustrum ovalifolium* with weak solutions of potassium permanganate CURTIS accelerated the development of roots (**2**). An attempt was made to test the possible value of potassium permanganate treatments with cotton cuttings. The bases of separate lots of cuttings were submerged with 2, 1, 0.5, and 0.25 per cent. solutions of potassium permanganate in water for thirty minutes before being placed in the propa-

gators. A similar lot of cuttings was treated with tap water for the same length of time. Calluses were developed on 68.6 per cent. of the water treated cuttings while 31.4 per cent. of calluses was the highest for any of the chemically treated group. To measure the general favorableness for callusing within the propagators used for the chemically treated cuttings, a number of untreated *Ligustrum japonicum* cuttings was introduced. That conditions were favorable for *Ligustrum* is indicated by the fact that 96.0 per cent. of these cuttings callused.

One factor concerning the propagators themselves was investigated. The standard number of cuttings used was ten per propagator but in one group 20 cuttings each were used. The percentages of cuttings callusing were 37.8 and 48.5 respectively for the 10 and 20 cuttings per propagator lots. Thus there appears to be no reason for not filling each propagator to capacity.

Of equal importance to any other factor is the medium in which cuttings are callused (**1, 3, 9**). For this purpose soil materials are extensively used, particularly, clean, sharp sand. The soil materials tested in this experiment included fine gravel, coarse sand, fine sand and Houston black clay. From table III it is evident that Houston black clay gave the best results. Cuttings placed in Houston black clay callused in 57.4 per cent. of the cuttings, while coarse sand, fine gravel, and fine sand induced callusing of 45.2, 35.9 and 15.8 per cent. respectively.

The volume of the soil in the propagator affects the result. When the propagators were filled with 200 milliliters of soil, 43.5 per cent. of the cuttings callused. With 350 milliliters of soil 35.1 per cent. callused. The use of 500 milliliters of soil seemed to interfere with callusing as only 12.5 per cent. of the cuttings used with this volume of medium callused. The volume of medium used was probably of little significance in itself but it is very likely that it influenced two other more important factors. When too little medium was used, the supply of moisture was probably lowered. With the use of too much medium the air space (available oxygen) was restricted. These two factors are very vital to the callusing of the cutting.

Although Sphagnum moss is usually highly recommended for callusing cuttings, we obtained very poor results with it. Not a single cutting callused where Sphagnum was used. Before the experiment had progressed very far, the poor results with Sphagnum moss were noted, and additional trials were made. Although various arrangements of cuttings in Sphagnum were tried, few cuttings were successfully callused.

Three separate lots of cotton cuttings were placed in propagators with no medium other than air. In one lot the cuttings and the interior of the propagator were washed with tap water. In another only the propagator

was washed, and in the third lot no free water was introduced. The results presented in table III clearly show that these were all less successful than the tests in which soil materials were used. Of the three series of propagators in which no solid medium was used the one in which no water was used gave the best results. In this lot 19.0 per cent. of the cuttings callused.

Temperature is one of the most vital factors affecting the callusing of cuttings (**7, 1, 3, 9**). One lot of cuttings was held at an average temperature of 11.5° C.; none of these callused. Three other lots were held at an average temperature of 16.3, 17.3 and 20.2° C., respectively. The percentage of cuttings callusing increased with increasing temperature, as is shown in table III.

Summary

Reviewing the results of the experiment on callusing cotton stem cuttings in glass fruit jar propagators, it is found that certain conditions provided were injurious. The use of soft wood cuttings was notably unsuccessful. The treatment of cotton cuttings with aqueous solution of potassium permanganate reduced the percentage of callusing in the concentrations tried. Spaghnum moss was found to be a very poor medium for cotton cuttings and only very few cuttings were callused without the use of soil media. An average temperature of 11.5° C. was too low to permit callusing.

Other conditions provided seemed to be optional. Cotton cuttings callus just as readily, and perhaps more so, after being stored for twelve hours as when used immediately. From the results obtained, it seems that smooth cuts are not entirely essential and that crowding of cuttings in the propagator is not injurious.

On the other hand several factors seem to be highly favorable in callusing. Mature wood gave results superior to medium mature and soft wood. The exposure of the base of the inverted cutting to the moist air above soil materials appears to provide good conditions for callusing. Houston black clay was more favorable to callusing than other soil material used whether the bases of the cuttings were covered or exposed. The highest temperature used (20.2° C.) produced the highest percentage of callusing.

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EXPLANATION OF PLATE

A. Cutting wood used: 1. Soft. 2. Medium mature. 3. Mature. 4. The main stem of a cotton plant as trimmed in the field. 5. A defoliated cotton plant showing lateral branching.

B. Types of cuttings as to maturity of wood: 1. Standard length mature. 2. Medium, and 3, Soft wood cuttings.

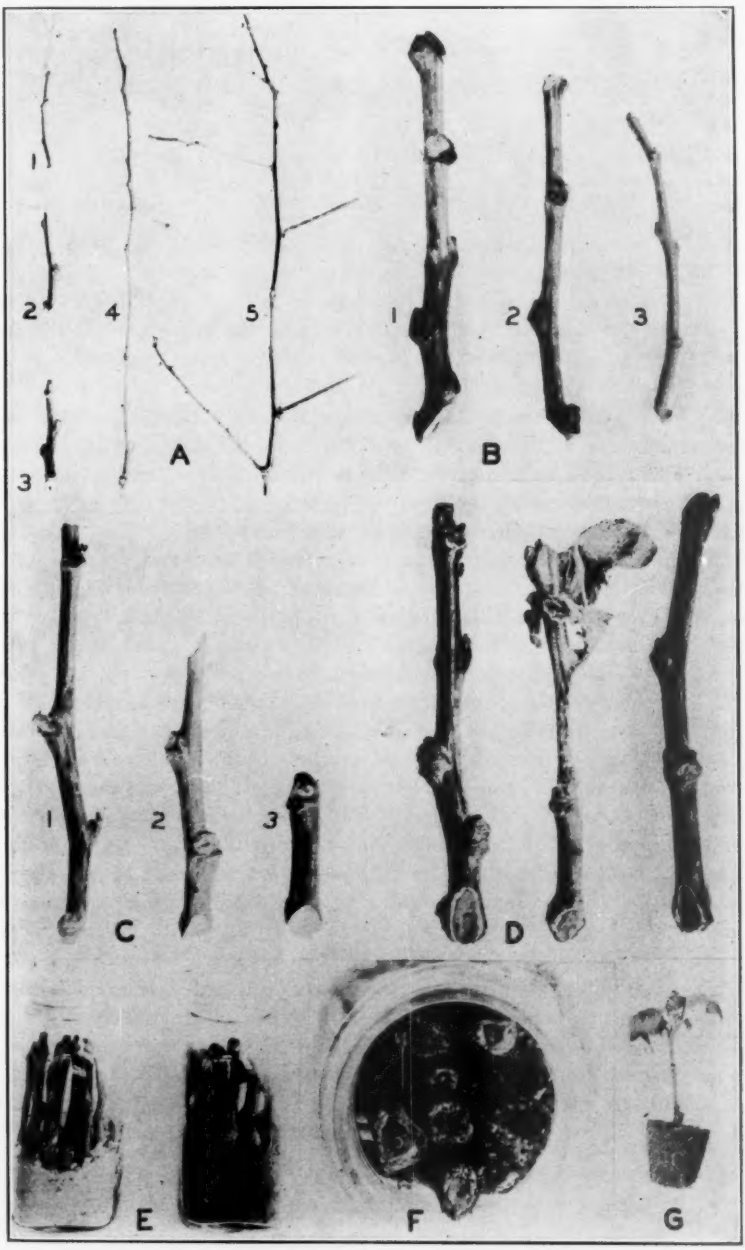
C. Other types of cutting used: 1. Rough trimmed. 2. Smooth cuttings of standard length. 3. Short two-node cuttings.

D. Three cuttings with typical callusing of cambium along basal cut.

E. Two jars showing placement of standard cuttings in the propagating cases.

F. The calluses of cuttings inverted in the jar with basal cuts exposed.

G. A thoroughly sorted cotton cutting.



REA—CALLUSING OF COTTON

PRELIMINARY STUDY OF ELONGATION OF ROOTS OF GEORGIA COLLARDS AS AFFECTED BY SODIUM LUMINAL[1]

T. W. Pratt

(WITH THREE FIGURES)

No study has hitherto been reported in botanical literature as to the toxic effects of sodium luminal on plant growth. Apparently, all of the work with sodium luminal of biological character which has been reported, with the exception of medical cases, has been based upon animal experimentation. Interest in phytopharmacology and phytotoxicology has been stimulated by the experiments of Macht and Krantz (7) who determined the effect of digitalis solutions on the growth of seedlings. They found that the inhibition of the root-growth of *Lupinus albus* was proportional to the concentration of the digitalis solutions employed.

Obviously, much information may be obtained by observing the behavior of plant tissue under the influence of sodium luminal. Since, however, no work on plant behavior in connection with sodium luminal, or luminal has previously been reported, it was considered advisable to simplify any problem selected and to eliminate all complicating factors possible, if practical. Georgia collards, a variety of *Brassica oleracea*, were chosen for the tests. They were the same kind of plants used by Farr (2) in his studies of the effect of solutions on the elongation of root hairs. The seed of Georgia collards was purchased from W. Atlee Burpee Co., Philadelphia, which company attested the purity of the seed.

The main objects of the work here reported are: (1) the observation of any physiological reactions on the elongation of the roots of Georgia collards as affected by sodium luminal; and (2) the determination of any correlation between the root elongation of the seedlings used in the experiments and the concentration of sodium luminal.

Procedure

The apparatus for the experiment was modified from that used by Farr (2) and set up as shown in fig. 1. Six microscopes, fitted with eyepiece micrometers calibrated in microns, were fixed rigidly on a platform placed on the bases of two iron supports. The barrels of the microscopes were arranged in a horizontal plane in order to allow the attachment of a glass water chamber to each stage.

[1] Contribution from the Department of Botany, University of Oklahoma, n.s., no. 5.

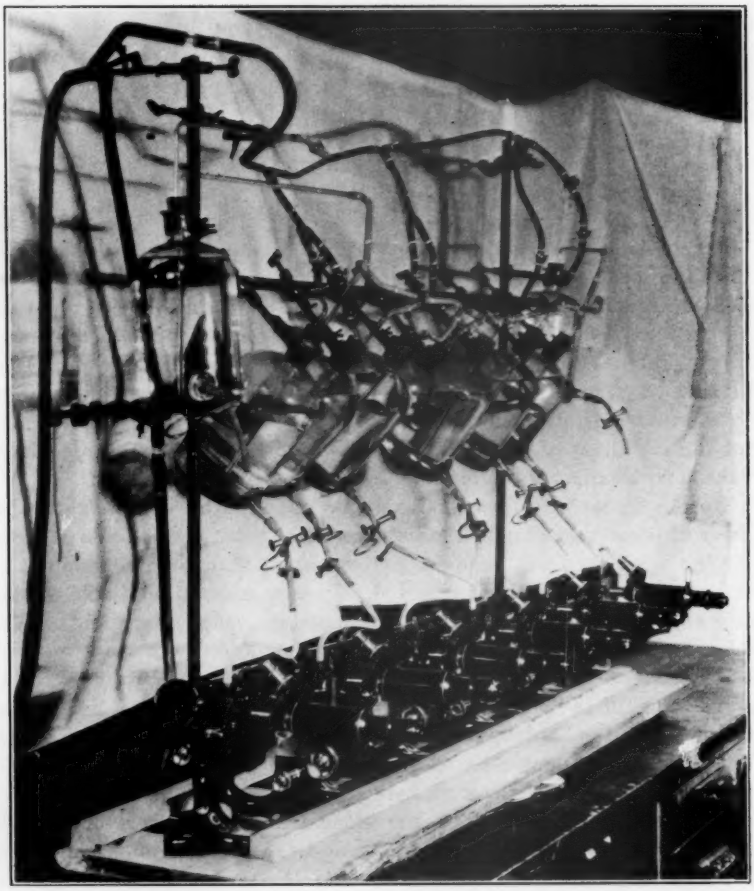


FIG. 1. Apparatus for the study of elongation of roots of Georgia collards as affected by sodium luminal.

De Khotinsky's hard cement was employed to cement together the margins of six water chambers whose inside dimensions were, approximately, $5.5 \times 5 \times 0.6$ cm. An inlet and outlet for solutions, consisting of glass tubing, were placed in the top of each chamber. A water chamber was held in any desired position on the microscope stage by a burette clamp and a 0.5 inch rubber band.

Six seedling holders, one to fit into each of the water chambers, were constructed. Each approximated 0.5 cm. in thickness and was made from

3 × 1 inch micro-slides. A small groove, about 3 mm. in width, was provided for in the holders to help keep the seedling in vertical position for measurements.

An aspirator and KOH tower were improvised to control the oxygen and carbon dioxide content of the solutions. The aspirator was made in the following manner. Two pieces of rubber tubing, each about four feet in length and having an inside diameter of 0.75 inch, were attached to two connections of a glass T-joint. This T-joint was mounted near the top of a support by a burette clamp in such a way that the third connection to the aspirator bottles and KOH tower was in an elevated position. One of the four-foot lengths of rubber tubing was joined with a water line; the other rubber tubing of similar length led into a drain trough.

A Squibb separatory funnel, 500-ml., served as a KOH tower. It was mounted near the center of the apparatus to facilitate the distribution of the CO_2-free air to all of the solution bottles. To the separatory funnel was attached a thistle tube for the removal of waste.

One system of connections, consisting of rubber tubing, glass T-joints, glass Y-joints, metal stopcocks, and bent glass tubing of suitable lengths, led from the aspirator to the eight 1500-ml. aspirator bottles and to the KOH tower. During operation of the aspirator, the CO_2-free air was drawn from the separatory funnel, containing potassium hydroxide, to the bottles containing solutions, the rate being controlled by the adjustment of stopcocks and the rate of water flow through the aspirator.

Six aspirator bottles, one for each microscope, were connected to the water chambers by rubber tubing. Hoffman (screw compressor type) clamps were used to control the gravity flow of the solutions from the aspirator bottles to the water chambers. Glass tubing and a small funnel, placed beneath the outlet of the water chamber, carried the used solution to a drain trough. Two additional aspirator bottles, connected with the aspirator and KOH tower, were used for the aeration of distilled water oftentimes required for solutions.

In general, the experimental routine was as follows. About 50 seeds of Georgia collards were placed on a wet filter paper in a moist chamber and permitted to germinate, in preparation for a day's tests. Two days afterwards, distilled water was added to the aspirator bottles and aerated with CO_2-free air one or more hours before the seedlings were mounted. Six seedlings, having roots which were straight and near 10 mm. in length, were selected and attached individually to the seedling holders with beeswax. Each water chamber was washed out and filled from the aspirator bottle by the solution in which a seedling was placed. The seeds of the young plants were allowed to remain above the water level. The root tip of each seedling was then focussed under a low power lens and the water

chamber made fast to the stage of the microscope with a burette clamp. Frequently the water chambers were changed in position to hold the root tips in the fields of vision of the microscopes. If no growth occurred in one or two hours, the mount was replaced or discarded. Only a few seedlings were necessarily remounted.

Usually careful observation of the seedlings was made between 8:00 and 11:00 P. M. One to three controls were selected for each day's tests. Readings of the elongation of the roots of all the seedlings were made every 15 minutes for 3 hours. Occasionally a reading was not recorded if some interference was evident. During the first hour of the 15-minute readings, 200 cc. of each concentration of sodium luminal required, was prepared. Five minutes before the expiration of the first hour of regular observation, the flow of distilled water was stopped. The remainder of the distilled water, in those aspirator bottles which were to be used for the drug, was replaced by a sodium luminal solution. At the end of the hour after readings were recorded, the water chambers were washed out by a rapid flow of solution from the six aspirator bottles, after which the flow was adjusted to drop regularly.

Readings of root elongation of the controls, as well as of the seedlings subjected to sodium luminal, were taken for 1.5 hours. Five minutes before the completion of the above 1.5 hour period of time, the delivery tubes from the aspirator bottles were again clamped. The aspirator bottles containing the sodium luminal solutions were washed and supplied with aerated distilled water. After the customary procedure, all of the water chambers were flushed with distilled water. Readings were further recorded for 2 15-minute periods. On completion of a day's observation, the readings which were at first designated in terms of the divisions of the eyepiece scale were then converted into microns and recorded. The average elongation of the roots of all the seedlings was tabulated as shown in table I.

The general routine was slightly modified as the plan of the procedure required. The work was divided into 3 series, each part consisting of the tests performed with certain concentrations of sodium luminal. Each solution of the drug was expressed in per cent. as was done by MACHT and KRANTZ (7) and arranged thus: Series 1—0.2, 0.4 and 0.8 per cent; Series 2—0.1, 0.5 and 1.0 per cent. and Series 3—0.3, 0.6, 0.7 and 0.9 per cent. In connection with each series, controls were used for comparison, two or three water chambers being reserved for that purpose.

The rate of the elongation of the roots of 10 seedlings was observed in each concentration of sodium luminal. This study, including the arrangement of the apparatus, was started in December, 1929, and finished in May,

TABLE I

AVERAGE ELONGATION OF ROOTS OF GEORGIA COLLARDS DURING 15-MINUTE INTERVALS FOR A PERIOD OF 3 HOURS IN DISTILLED WATER AND SODIUM LUMINAL

ELONGATION OF CONTROL IN DISTILLED WATER												
μ 133.1	μ 138.3	μ 139.5	μ 137.7		μ 138.0	μ 143.8	μ 144.2	μ 144.2	μ 146.6	μ 137.6	μ 137.3	μ 134.2
DISTILLED WATER				CONCENTRATION	SODIUM LUMINAL						DISTILLED WATER	
179.4	172.9	185.7	167.9	0.2 per cent.	101.5	77.6	64.1	60.2	38.0	35.4	154.5	96.1
169.3	162.6	166.7	171.3	0.4 per cent.	75.1	59.9	41.9	25.4	14.6	5.1	53.6	25.6
169.4	174.5	185.9	193.6	0.8 per cent.	53.4	29.2	6.3	3.2	–9.0	-25.4	–2.4	12.9
178.7	187.0	190.2	213.1	0.1 per cent.	123.0	106.5	107.8	110.3	88.6	87.2	170.4	133.7
180.3	185.4	190.2	209.5	0.5 per cent.	72.7	49.8	40.8	39.5	15.2	26.5	137.6	81.7
177.0	181.1	184.7	196.2	1.0 per cent.	29.3	21.6	10.2	7.6	–0.6	–5.7	67.1	16.9
143.8	161.0	158.5	196.4	0.3 per cent	71.5	43.9	63.5	35.3	40.7	53.9	169.6	131.3
154.1	159.3	165.6	192.5	0.6 per cent.	48.9	49.7	42.0	25.4	19.0	25.5	131.8	65.1
121.9	180.2	138.1	190.0	0.7 per cent.	42.2	28.1	18.9	28.1	13.9	11.8	124.3	63.3
192.4	138.1	141.1	151.1	0.9 per cent.	17.8	22.8	12.6	3.1	4.4	4.8	93.3	29.5

TABLE II

AVERAGE RATE OF ELONGATION OF ROOTS OF GEORGIA COLLARDS GROWN AT FIRST IN DISTILLED WATER, THEN IN SODIUM LUMINAL, AND AGAIN IN DISTILLED WATER

EXPERIMENTS	ELONGATION IN DISTILLED WATER					ELONGATION IN SODIUM LUMINAL SOLUTIONS				ELONGATION ON APPLICATION OF DISTILLED WATER FOLLOWING SODIUM LUMINAL[3]	
	DURATION OF APPLICATIONS				COMPARATIVE ELONGATION	CONCENTRATION USED	DURATION OF APPLICATION		COMPARATIVE ELONGATION		
	1 HOUR	AVERAGE, 15 MINUTES	1.5 HOURS	AVERAGE, 15 MINUTES			1.5 HOURS	AVERAGE 15 MINUTES		FIRST 15 MINUTE PERIOD	SECOND 15 MINUTE PERIOD
	μ	μ	μ	μ	*per cent.*[1]	*per cent.*	μ	μ	*per cent.*[2]	*per cent.*	*per cent.*
Control ...	548.72	137.18	854.67	142.44	103.83						
1 ...	769.22	192.30	.....	.....	.....	0.1	799.5	133.25	69.29	88.56	69.55
2 ...	706.0	176.5	.....	.....	.....	0.2	378.8	63.13	35.58	87.50	54.49
3 ...	659.83	164.95	.....	.....	.....	0.3	309.02	51.5	31.27	102.81	79.59
4 ...	670.0	167.5	.....	.....	.....	0.4	223.8	37.3	22.27	32.03	15.27
5 ...	765.6	191.4	.....	.....	.....	0.5	286.52	47.75	24.94	71.89	46.68
6 ...	671.5	167.87	.....	.....	.....	0.6	210.76	35.1	20.9	78.51	38.82
7 ...	630.25	157.56	.....	.....	.....	0.7	143.27	23.87	15.14	78.89	40.21
8 ...	723.2	180.8	.....	.....	.....	0.8	58.7	9.78	5.409	−1.36	7.17
9 ...	562.86	140.7	.....	.....	.....	0.9	65.58	10.93	7.75	66.33	21.01
10 ...	759.24	189.81	.....	.....	.....	1.0	56.24	9.37	4.92	35.87	8.93

[1] The average rate for 15 minutes in column 3 is considered the basis of comparison = 100 per cent. elongation. Columns 4, 5 and 6 show with distilled water, how the sodium luminal effects are calculated.

[2] The per cent. of elongation is determined as the ratio of the average elongation in sodium luminal for 15 minutes, to the elongation in water for 15 minutes, in column 3. Thus $133.25 \times 100 \div 192.3 = 69.29$ per cent.

[3] These percentages are also based upon the averages of column 3, which are made the standard of comparison (= 100 per cent.)

1930. The readings, however, were taken over a period from March 3, 1930 to April 21, 1930.

Results

In table I, the control is based on the readings of all the seedlings in the different series not affected by sodium luminal. The percentages represent the different concentrations of sodium luminal employed, and their arrangement in the table is according to the order followed in the different series. The average elongation of the roots in microns, as recorded in the first four columns of the treated seedlings, was made in distilled water; the figures listed in columns 6, 7, 8, 9, 10, 11, dealing with the same plants, represent the growth as influenced by different strengths of the drug, and the averages in the last two columns of table I stand for the elongation observed during the second application of distilled water.

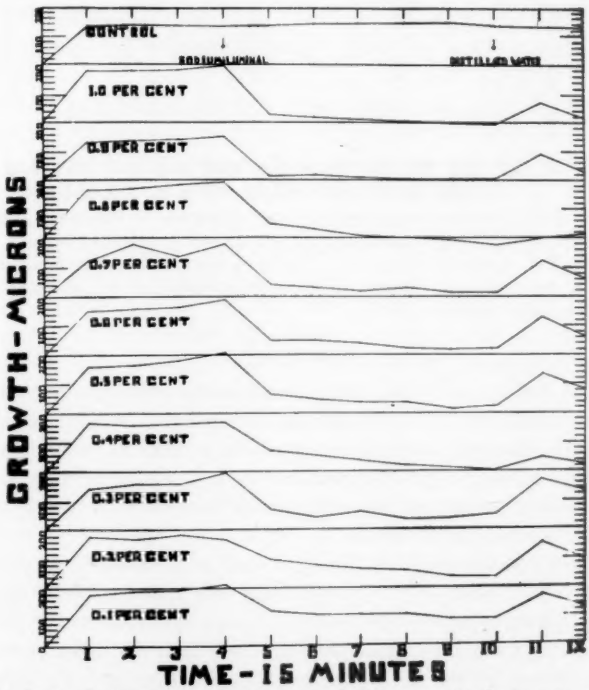


FIG. 2. Rate of elongation of the roots of Georgia collards in microns for 15-minute intervals during a period of 3 hours. Curves plotted from the averages of root-elongation taken from table I. Divisions on the ordinates represent elongation in microns; abscissas, corresponding with the bases of the graphs, are divided into equal parts which represent 15-minutes intervals of time.

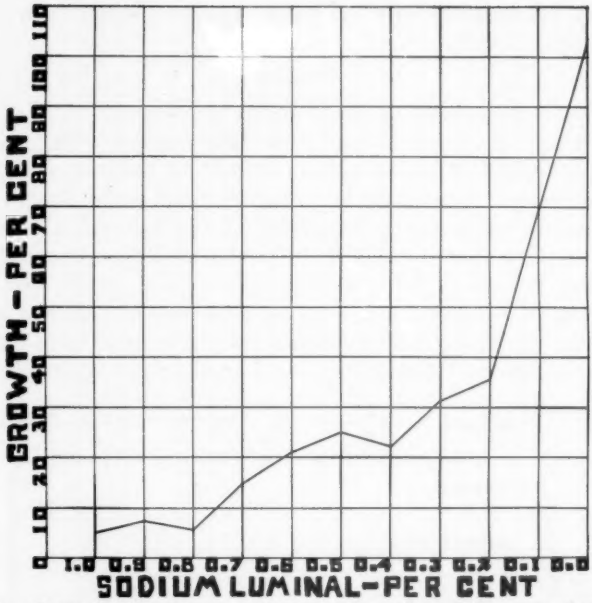


FIG. 3. Curve showing the per cent. of the elongation of the roots of Georgia collards grown in sodium luminal as compared with growth in distilled water. From data of column 10 and column 6 in table II. The percentages of elongation are represented by points on the ordinates. Spaces on the abscissas indicate the per cent. concentrations of the sodium luminal.

Discussion

Distilled water was found to be practical for use as a culture solution in these experiments. A few tests demonstrated that sodium luminal could not be used in Knop's nutrient solution as a precipitate would form when the two were mixed. Since the roots of Georgia collards made reasonably constant elongation in distilled water for a few hours, a nutrient solution was not considered necessary.

It is well known that distilled water is not suitable, in general, for plant cultures. TRUE (**10**) reported that the radicles of *Lupinus albus* were very sensitive to distilled water. TRUE and BARTLETT (**12**) found that the roots of *Lupinus albus* gave up their salts until the reserves were exhausted, when grown in the dark in distilled water. In referring to the effect of distilled water on the roots of certain seedlings, OSTERHOUT (**8**) states that "The effects which have been described as due to distilled water were also produced by water taken directly from ponds, rivers, springs; they are not due, therefore to toxic substances resulting from the

process of distillation." However, TRUE (**10**), in alluding to a paper published by TRUE and BARTLETT (**11**), claimed that Canada field peas make a fairly healthy growth in distilled water, which indicates that not all plants are sensitive in the same degree to distilled water.

The results of experiments conducted by HIBBARD (**3**) indicate that the seedling roots of *Lupinus albus* excrete a substance that inhibits growth. To prevent the possibility of such excretions modifying the behavior of the roots of Georgia collards, a flowing solution, which would remove toxic substances, was employed.

Due to the fact that a nutrient solution could not be used satisfactorily in connection with sodium luminal, root hairs were eliminated as possible test objects. Only relatively few plants, according to FARR (**2**) produce root hairs in solutions. Although Georgia collards will produce aquatic root hairs in a well-balanced nutrient solution, no similar development occurs in distilled water. Actually, there is a tendency for the amphibious root hairs of Georgia collards to become abortive when immersed in distilled water.

The use of root hairs would no doubt offer some advantages in determining the effects of certain drugs on plant growth. Root hairs are very sensitive and development is relatively simple, as explained by FARR (**1**). JEFFS (**4**) who investigated the relation of the elongation of root hairs to light and temperature, recognized that the response of root hairs could be more accurately measured than the complex results occurring in a multicellular structure such as a root.

Acceptable work, however, has been accomplished by the action of various chemical agents on the retardation of root growth. TREALEASE and TREALEASE (**9**) claim that root elongation in very young seedlings is not too complex for reliable, experimental results. In addition, MACHT and KRANTZ (**7**) obtained most consistent results in demonstrating that the root growth of the seedlings of *Lupinus albus* was inversely proportional to the concentration of the solutions of digitalis used.

No provision was made for the control of temperature. Variations in temperature may cause retardation or acceleration of root elongation. LEITCH (**6**) reported that the rate of growth of the roots of *Pisum sativum* can be expressed as a uniform curve from $-2°$ to about 29°. Work performed by LEHENBAUER (**5**) as abstracted by TREALEASE (**9**) shows that VAN'T HOFF'S rule, that there is a doubling or trebling of chemical action by each rise in temperature of 10° C., applies to the growth of maize seedlings, within the range of 20 to 30° C.

The widest range of temperature (recorded about every hour) during the course of these experiments, was from 19.5 to 28° C. Such extremes of temperature were observed only twice. Usually the thermometer varied

from 22 to 24° C. Although controlled conditions would have been preferable, the fluctuations affect the seedlings equally in any given series of tests.

The disadvantages of working under variable light conditions were partially offset by employing three or more concentrations in each series. Any variations of root elongation of the plants within a series, based on a number of tests, are evidently due to the differences in the concentration of the sodium luminal.

No attempt is made to compare the effects produced by sodium luminal on root-elongation as shown in the different series, since influential factors such as temperature, light, humidity, etc., were not controlled.

The writer wishes to express his appreciation to Dr. R. E. Jeffs, plant physiologist, who ably directed the problem, and to acknowledge his indebtedness to Dr. Paul B. Sears, Head of the Department of Botany, who generously supplied the necessary materials, and offered many constructive criticisms.

Summary

1. Distilled water was found suitable as a plant culture for a preliminary study of the root-elongation of Georgia collards as affected by sodium luminal.

2. Sodium luminal, 0.1 to 1.0 per cent. was toxic to the roots of Georgia collards.

3. Pronounced retardation of root-elongation was produced by sodium luminal, used in concentrations from 0.1 to 1.0 per cent., within 1.5 hours after administration.

4. The effect of the sodium luminal on the root-elongation was immediate, the greatest retardation occurring during the first of the six 15-minute intervals of observation.

5. Distilled water partially removed the depressant action of the sodium luminal and accelerated the elongation of the roots.

6. In each series of experiments conducted, a close relation appeared between the elongation of the roots and the concentration of the sodium luminal. The stronger the concentration of the drug, the greater the retardation of root growth.

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EFFECT OF VARIOUS METHODS OF STORAGE ON THE CHLOROPHYLL CONTENT OF LEAVES[1]

PHILIP A. HARRIMAN

(WITH ONE FIGURE)

Problems involving the quantitative determination of the chlorophyll content of leaves have been carried out chiefly with plants grown in a greenhouse or in the immediate vicinity of a laboratory. This allows the extraction of the chloroplast pigments within a few minutes after the samples are secured and, as the chlorophyll content of the leaves is practically unaltered, the results may be taken to represent the conditions existing in the living tissue. A recent investigation by DEUBER (**1**) shows a wide variation in the chlorophyll content of the leaves of different species of trees. The chlorophyll determinations were all made from fresh leaves gathered from trees growing near the laboratory. It would be desirable to have similar information regarding the leaves of trees growing in other localities where environmental conditions are different. The investigation of SPRAGUE and SHIVE (**9**) on the relations between chloroplast pigments and the dry weights of tops in dent corn and the work of GUTHRIE (**3**) on the effect of environmental conditions on the chloroplast pigments suggest interesting and instructive problems that might be carried out with plants growing in widely separated habitats.

In such cases the extraction and determination of the pigments must be made from stored or preserved leaves. As the question of storage of green tissues for later determination of chloroplast pigments has received only slight attention, it is the purpose of the present investigation to determine the effect of various methods of storage on the chlorophyll content of leaves.

Procedure

About 50 experiments were carried out with duplicate and in some cases triplicate samples. Since only a limited number of determinations could be made at the same time, it was necessary to divide the experiments into a number of series. In each series one set of samples was used for immediate extraction to determine the chlorophyll content of the fresh leaves; another set of samples was used to determine the moisture content of the leaves; and the rest were dried or stored under the various conditions of the respective experiments. The chlorophyll content of the dried or stored leaves was then determined and the amount retained expressed in per cent. of the chlorophyll content of the fresh leaves. The determinations were all made

[1] Contribution from the Osborn Botanical Laboratories, Yale University.

on Wilson soy bean or on dwarf nasturtium leaves and, in most cases, on both.

The chloroplast pigments were extracted and separated after the method of WILLSTÄTTER (**10**) as modified by SCHERTZ (**7, 8**) and the amount of chlorophyll determined colorimetrically by means of a Duboscq colorimeter. The probable error of a single observation by this method is stated to be about 3.3 per cent. for chlorophyll solutions with concentrations of 0.05 grams per liter. In the present investigation the average probable error determined by Bessel's formula, P.E.M. equals $.6745\sqrt{\frac{\Sigma d^2}{n(n-1)}}$, is 2.16 per cent. Both pure chlorophyll[2] solutions and the artificial color standard of GUTHRIE (**2**) were used, but because of its greater stability it was found advantageous to use the artificial standard for most of the determinations.

To determine the effect of the temperature of storage on the chlorophyll content of the leaves, they were dried in ordinary drying ovens at 98° C., 72° C., 60° C., 45° C., 30° C., and at room temperatures; they were stored in an electric refrigerator at 5° C. and at −5° C.; and they were frozen with Dry Ice (solid carbon dioxide snow). In every case the leaves were spread on coarse filter paper in a single layer.

In order to bring about a rapid removal of the water from the leaves without increasing the temperature, a vacuum desiccator was used. The desiccator was evacuated by means of a filter pump and both anhydrous calcium chloride and concentrated sulphuric acid were used as dehydrating agents. A number of leaf samples were also desiccated at reduced pressure in the absence of oxygen and carbon dioxide, the desiccator being filled with air which had passed through a solution of potassium hydroxide and pyrogallic acid.

Discussion of results

EFFECT OF TEMPERATURE

When dried at 98° C. soy bean leaves retained only 30 per cent. of the chlorophyll found in the fresh leaves, but when dried at 72° C. they retained 88 per cent. of their chlorophyll. With further decreases of the temperature of drying, still higher percentages of the chlorophyll were retained, and at 45° C. 98 per cent. or practically all of the chlorophyll remained in the dried leaves. Soy bean leaves dried at 30° C., however, suffer a slight loss of chlorophyll and those dried at room temperature (approximately 20° C.) retain only 70 per cent. of their chlorophyll. It is of interest to note that leaves dried at this temperature retain less chlorophyll than those

[2] The pure chlorophyll used was obtained through Dr. CARL G. DEUBER from Dr. F. M. SCHERTZ of the Bureau of Plant Industry, U. S. Department of Agriculture.

dried at any other temperature except 98° C. In soy bean leaves stored at −5° C., at which temperature they were not actually frozen, only 81 per cent. of the chlorophyll was retained; but, when the leaves were frozen with Dry Ice, the chlorophyll content of the leaves was unaltered. See table I.

TABLE I

EFFECT OF TEMPERATURE OF DRYING ON CHLOROPHYLL CONTENT OF SOY BEAN LEAVES

SAMPLE NO.	TEMPERATURE	CHLOROPHYLL		
		AMOUNT PER GM. OF DRY WEIGHT		AMOUNT RETAINED
		FRESH LEAVES	DRIED LEAVES	
	°C.	mg.	mg.	per cent.
II A	98	22.5	6.8	30
X A	72	13.7	12.0	88
IX C	60	21.9	20.8	95
VI C	45	16.5	16.1	98
XII G	30	17.5	16.9	96
II B	20	22.5	15.8	70
II C	5	22.5	17.2	76
IX M	−5	21.9	17.7	81
XII M	−40 to−10	17.5	18.1	103

The results obtained with nasturtium leaves are similar to those obtained with the soy bean leaves and are given in table II. The maximum amount

TABLE II

EFFECT OF THE TEMPERATURE OF DRYING ON CHLOROPHYLL CONTENT OF NASTURTIUM LEAVES

SAMPLE NO.	TEMPERATURE	CHLOROPHYLL		
		AMOUNT PER GM. OF DRY WEIGHT		AMOUNT RETAINED
		FRESH LEAVES	DRIED LEAVES	
	°C.	mg.	mg.	per cent.
I A	98	21.8	7.1	32
IV A	72	23.8	18.8	79
VIII C	60	17.3	16.1	93
XIII C	45	18.0	15.5	86
VII G	30	17.7	15.7	88
I B	20	21.8	17.8	81
I C	5	21.8	17.4	80
XIII M	−5	18.0	15.7	87
VIII N	−40 to−10	17.3	17.5	101

of chlorophyll retained in the oven dried leaves is 93 per cent., somewhat less than the maximum amount found in the oven dried soy bean leaves. Furthermore, this maximum was found in the nasturtium leaves that were dried at 60° C. rather than 45° C. as was the case with the soy bean leaves. This is in agreement with the optimum temperature for drying plant tissues suggested by MURNEEK (6). The nasturtium leaves dried at room temperature retained about 10 per cent. more chlorophyll than did the soy bean leaves dried at the same temperature. A graphic comparison of the results obtained with the two kinds of leaves is shown in fig. 1.

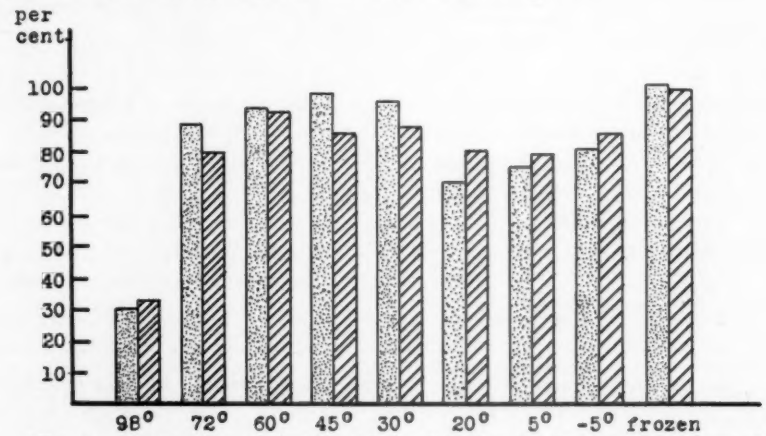

FIG. 1. A comparison of the chlorophyll retained in dried or stored soy bean and nasturtium leaves. The amounts of chlorophyll retained given in per cent. are taken from tables I and II.

soy bean. nasturtium.

EFFECT OF DESICCATION AT REDUCED PRESSURE

The results of the experiments to show the effect of desiccation at reduced pressure are shown in tables III and IV. Nasturtium leaves dried in an ordinary desiccator over calcium chloride at atmospheric pressure retained only 62 per cent. of the chlorophyll of the fresh leaves, but when desiccated at a pressure approximately equivalent to 27 cm. of mercury, 97 per cent. of the chlorophyll was retained. Leaf samples from the series that was desiccated at the same pressure and subsequently stored in an ordinary desiccator over calcium chloride retained 92 per cent. of their chlorophyll at the end of ten days, and 83 per cent. at the end of 20 days. This seems to indicate that the factors which cause the loss or destruction of the chlorophyll in nasturtium leaves are not entirely eliminated by this treatment. Similar results, however, were not obtained with the soy bean leaves

TABLE III

EFFECT OF VARIOUS METHODS OF DESICCATION ON CHLOROPHYLL CONTENT OF LEAVES

SAMPLE NO.	METHOD OF DESICCATION			SUBSEQUENT STORAGE OVER $CaCl_2$	CHLOROPHYLL RETAINED	
	DRYING AGENT	PRESSURE	TIME		SOY BEAN	NASTURTIUM
		cm. Hg	*days*	*days*	*per cent.*	*per cent.*
VII K	$CaCl_2$	76	12	0		62
III G	$CaCl_2$	27	3	0		97
III E	"	27	3	10		92
III F	"	27	3	20		83
V E	$CaCl_2$	27	5	0	89	
V G	"	27	5	10	89	
X E	H_2SO_4	7	4	0	98	
III H	$CaCl_2$	27	3	Dried at 98° C. 3 day		47

TABLE IV

EFFECT OF DESICCATING LEAVES AT REDUCED PRESSURE IN ABSENCE OF OXYGEN AND CARBON DIOXIDE

SAMPLE NO.	METHOD OF DESICCATION			SUBSEQUENT STORAGE IN ABSENCE OF O_2 AND CO_2	CHLOROPHYLL RETAINED	
	DRYING AGENT	PRESSURE	TIME		SOY BEAN	NASTURTIUM
		cm. Hg	*days*	*days*	*per cent*	*per cent*
VI E	$CaCl_2$	27	4	0	90	
VI F	"	27	4	10	90	
IV I	"	27	4	0		90
IV J	"	27	4	10		84

desiccated at this pressure. They retained 89 per cent. of their chlorophyll and none of this was lost on subsequent storage. Desiccated at a lower pressure, approximately equivalent to 7 cm. of mercury over concentrated sulphuric acid, soy bean leaves retained 98 per cent. of their chlorophyll. One set of samples, which was dried in an oven at 98° C. after it had been desiccated at reduced pressure, suffered a further loss of about 50 per cent. of its chlorophyll.

The nasturtium leaves that were desiccated at reduced pressure in the absence of oxygen and carbon dioxide retained 90 per cent. of their chloro-

phyll which is somewhat less than was retained by the same kind of leaves desiccated at the same pressure in air. When stored in an atmosphere free from oxygen and carbon dioxide for a period of ten days, they suffered a further loss of chlorophyll.

The results obtained with the soy bean leaves desiccated in the absence of oxygen and carbon dioxide are but slightly different from those obtained with the same kind of leaves desiccated in air at the same pressure. They retained 90 per cent. of their chlorophyll and this was unaltered after being stored for 10 days in the same atmosphere.

Factors contributing to the loss of chlorophyll

Although the purpose of the present investigation is to determine the extent to which the chlorophyll is lost in stored leaves rather than to determine the factors which are effective in bringing about this loss, certain of the results obtained are sufficiently suggestive to warrant some speculation as to these causes.

Since only 30 per cent. of the chlorophyll was retained in the leaves dried at 98° C., it seems apparent that this temperature is sufficiently high to bring about the decomposition of the pigment. This is also borne out by the fact that the extracts from these leaves were of a dirty brown appearance rather than the clear green color of pure chlorophyll solutions. Leaves that were desiccated at reduced pressure for three days retained 97 per cent. of their chlorophyll, but when subsequently dried at 98° C., only 47 per cent. was retained. This also indicates that high temperatures are destructive to chlorophyll, but to a less degree if the leaves are practically free from water.

At lower temperatures, however, other factors are probably responsible for the loss of chlorophyll. Link (**4**) found that a drying temperature below 65° C. did not check enzymic activity in green succulent tissue. It is stated by Link and Tottingham (**5**) that even in a vacuum oven metabolic changes are inhibited only by a higher temperature (80° C.). The optimum temperature for the action of chlorophyllase as stated by Willstätter (**10**) is about 20° C. Either an increase or a decrease in the temperature will retard the activity of the enzyme and the amount of chlorophyll lost due to this factor will be less. It will be noted that with both the soy bean and nasturtium leaves less chlorophyll was retained in those dried at 20° C. than in the leaves dried at any other temperature (except 98° C.). The amount of chlorophyll retained in the dried leaves increased whether the temperature of drying was increased or decreased. When the leaves were frozen, enzymic activity was probably entirely stopped and the leaves stored in that condition retained all of their chlorophyll. Guthrie

(3) also found that frozen leaves retained all of their chlorophyll, but that the ratio between the components (*a* and *b*) was altered. Other factors, such as light and the presence of oxygen and carbon dioxide, may have some effect on the chlorophyll content of stored leaves, but the chief factors contributing to the loss of chlorophyll seem to be temperature and enzymic activity.

Summary and conclusions

Soy bean and nasturtium leaves were dried at various temperatures and desiccated at reduced pressure; they were stored at low temperature in a refrigerator and frozen with Dry Ice. The chlorophyll content of the leaves was determined in the fresh condition and after drying or storage. The amount of chlorophyll retained in the leaves was determined and expressed in terms of per cent. of the chlorophyll content of the fresh leaves. In some cases the results obtained were quite different for the two kinds of leaves used. This would indicate that one might expect still different results if other kinds of leaves were used. For this reason it does not seem wise to draw too general conclusions from the results obtained. The following points are given as a summary of the results obtained with the soy bean and nasturtium leaves.

1. Leaves dried at high temperatures (98° C.) suffer a considerable loss of chlorophyll (approximately 70 per cent.).

2. Leaves dried at room temperatures (18° C. to 24° C.) lost 20 to 30 per cent. of their chlorophyll.

3. The optimum temperature range for oven drying seems to be from 45° C. to 60° C., but even at these temperatures some chlorophyll may be lost.

4. Low temperatures are ineffective in preventing the loss of chlorophyll unless the leaves are actually frozen.

5. Leaves frozen with Dry Ice retain all of their chlorophyll.

6. Leaves desiccated at reduced pressure (7 cm. mercury) over concentrated sulphuric acid at room temperature lost little or no chlorophyll.

7. Leaves desiccated at reduced pressure in the absence of oxygen and carbon dioxide lost more chlorophyll than leaves desiccated in air at the same pressure.

From these results it seems apparent that freezing is the most satisfactory method of storing leaves for later quantitative determination of the chlorophyll. Leaves desiccated at reduced pressure also retain all or nearly all of their chlorophyll, but this procedure is possible only in a laboratory where the extraction and determination of the chlorophyll could also be carried out directly with the fresh leaves.

The writer wishes to express his appreciation to Dr. CARL G. DEUBER who suggested the problem and under whose direction the work was done.

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EFFECT OF FERTILITY ON THE CARBOHYDRATE-NITROGEN RELATION IN THE SOYBEAN[1]

F. A. Welton and V. H. Morris[2]

(WITH THREE FIGURES)

Introduction

In connection with a study of the cause of lodging in soybeans as grown in corn for silage it was found that the shading by the corn resulted in a reduction of the percentage of carbohydrates and total dry matter, and that the stems of soybeans thus developed were relatively soft, pliable, and weak. In cereals like oats and wheat a similar situation in the stems with consequent lodging has been found to develop as a result not only of shading but also from hyper-nutrition. In the latter case, particularly in the presence of an abundance of nitrates accompanied by suitable moisture relations, there was set up within the oat and wheat plants a low carbohydrate-nitrogen relation characterized by a preponderance of vegetative growth, soft, pliable stems, and consequent lodging. Conversely, under relatively poor soil conditions there was developed a high carbohydrate-nitrogen relation characterized by a preponderance of reproductive growth, hard, rigid, stiff stems, and non-lodging.

Object

The purpose of the work presented in this paper was to ascertain if hyper-nutrition, particularly an abundance of nitrates, affects the carbohydrate-nitrogen relation in soybeans, a legume, the same as it has been shown to affect the carbohydrate-nitrogen relation in other plants such as oats, wheat, and tomatoes.

Material and methods

Soybean plants were grown in square-yard areas on poor, medium, and rich soils. The poor soil was made by mixing one part of Wooster silt loam with three parts of common creek sand which had previously been passed through a sand screen in order to remove stones and any coarse foreign material. The medium soil consisted of Wooster silt loam which was in a good state of fertility. The rich soil was prepared by mixing with one part of the loam three parts of well rotted manure. These three classes of soil were placed side by side and separated from each other by boards one inch thick and seven inches wide. They are henceforth designated in this paper as sand-soil-manure plots.

[1] Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associates in Agronomy.

Soybeans were planted in these plots in 1924 and 1925. In 1924 seeds of the Elton variety were planted June 11, putting two seeds in hills spaced one foot apart each way. Samples for analysis were taken September 18 when the plants were well developed, but before the leaves had begun to drop. In 1925 seeds of the Hamilton variety were sown May 29, and samples for analysis were taken September 21.

In sampling, the leaves were removed and the lower two-thirds only of the stems were used. These were cut into pieces one-half to three-fourths of an inch long, placed in wide-mouthed, glass-stoppered bottles, covered with alcohol, the strength of which, after allowing for the moisture contained in the plants, was about 70 per cent., and then heated about an hour at approximately 78° C. The samples were then set aside and allowed to stand for two or three months before analysis.

Aside from total dry matter, analyses were made for certain of the constituents of which the dry matter is composed, namely, free reducing sugars, inverted sugars, easily hydrolyzable carbohydrates, cellulose, and lignin.

Results

The results of the analyses of the plants grown on the three grades of fertility sand-soil-manure in the two seasons, 1924 and 1925, were as shown in the accompanying table.

From the table it may be seen that as the fertility of the soil increased, the dry matter and the total carbohydrates of the soybean stems decreased. Comparing the plants grown in the sand with those grown in the manure, the stems of the former contained more free reducing sugars, more easily hydrolyzable carbohydrates, more cellulose, and more lignin. As far as

TABLE I

EFFECT OF FERTILITY ON COMPOSITION OF SOYBEAN STEMS

MATERIAL	1924			1925		
	SAND	SOIL	MANURE	SAND	SOIL	MANURE
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Moisture	70.2	72.2	73.2	61.2	63.2	72.0
Dry matter	29.8	27.8	26.8	38.8	36.8	28.0
Total nitrogen	0.7	0.5	0.6	0.4	0.4	0.4
Free reducing sugars	1.0	1.1	0.7	1.4	0.8	0.5
Inverted sugars	0.3	0.3	1.2	0.1	1.5	1.3
Easily hydrolyzable carbohydrates	10.2	10.9	8.8	8.2	9.3	6.2
Cellulose	4.3	3.3	2.9	3.8	3.3	2.5
Lignin	9.8	9.1	7.5	17.6	15.4	10.8
Total carbohydrates	25.6	24.7	21.1	31.1	30.3	21.3

these constituents are concerned, the results are in agreement with those found in certain non-leguminous plants, the tomato (1) and oats and wheat (3) when the latter were grown under similar environmental soil conditions. In the soybean, however, the increase in carbohydrates was not accompanied by a simultaneous decrease in nitrogen as was found to be the case in the non-legumes already mentioned.

The dissimilar response of the soybean may be due to the development of nitrogen-fixing bacteria which live in a symbiotic relationship in the nodules on the roots. LEONARD (2) found that nodule formation in soybeans is closely related to the carbohydrate-forming function of the plant. Since the nitrogen-fixing bacteria are dependent on the host for available carbohydrates as a source of energy, the larger the supply of carbohydrates, the more favorable are the conditions for the fixation of free nitrogen. It may, therefore, be expected that when soybeans are grown under certain conditions, such as in a poor soil, the percentage of nitrogen in the tissues will more nearly approach that found when the plants are grown under conditions which tend to inhibit the accumulation of carbohydrates as in the case of soil high in nitrates. The plants grown in the sand in 1925 showed percentages of nitrogen as high as, and in 1924 higher than, those grown in the manure. Apparently this was made possible through the relatively greater development of roots and nodules in the plants grown in the sand, as shown in table II.

TABLE II

EFFECT OF TYPE OF SOIL, SAND-SOIL-MANURE, ON THE WEIGHT OF SOYBEAN PLANTS, INCLUDING ROOTS, AND NUMBER OF NODULES (7 PLANTS)

KIND OF SOIL	GREEN WEIGHT		PROPORTION OF ROOTS	NUMBER OF NODULES
	TOPS	ROOTS		
	gm.	*gm.*	*per cent.*	
Sand	455	30	6.2	379
Soil	508	33	6.1	237
Manure	745	26	3.4	62

The proportion of roots was nearly twice as high in the plants grown in the sand as in those grown in the manure. The growth of tops, however, was relatively small and the stems, like those of the non-legumes to which reference has already been made, were comparatively hard and rigid and not inclined to lodge. The relative size of roots and number of nodules are illustrated in the accompanying figures 1, 2 and 3.

Further evidence to the effect that low fertility does not necessarily result in a low nitrogen content of the soybean was afforded by the analyses

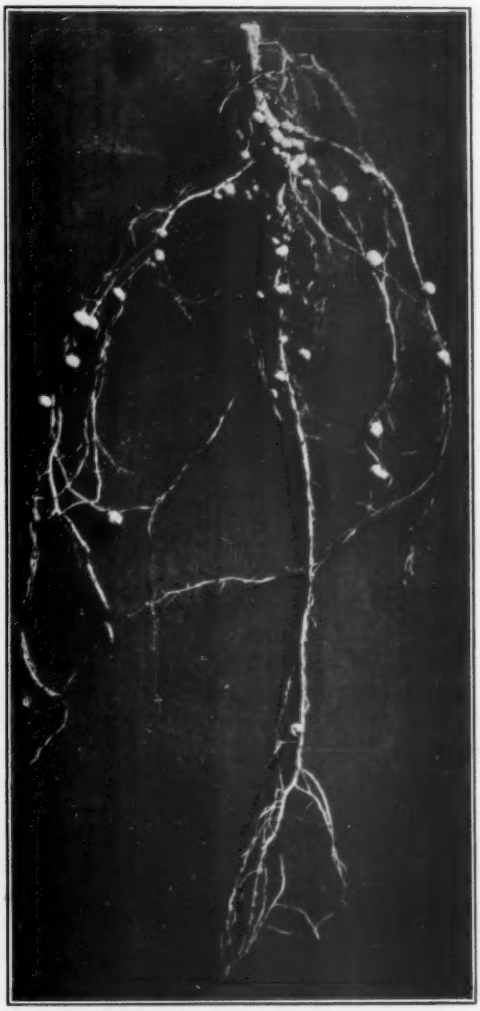

FIG. 1. Root system of soybean grown on sand. Nodules very abundant.

of plants grown in sand and manure in the summer of 1928. Both soluble and insoluble nitrogen determinations were made separately on the leaves and the entire stems. The results obtained were as shown in table III.

Moreover, the kind of soil on which soybeans are grown apparently has little or no effect on the type of nitrogen compounds in the stems and leaves for the percentages of soluble and insoluble nitrogen in the plants grown in

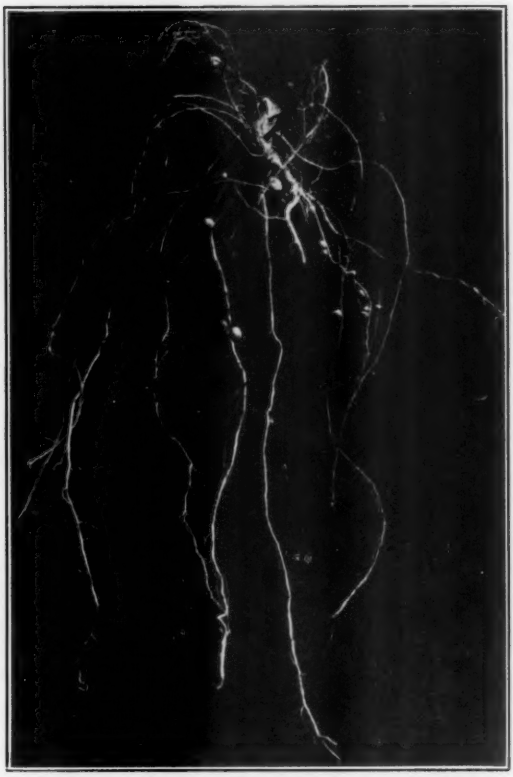

FIG. 2. Root system of soybean grown on soil. Nodules less abundant.

the sand were practically the same as were those in the plants grown in the manure.

Conclusions

Soybeans grown in sand (3 parts of sand to 1 of Wooster silt loam) contained more dry matter and more total carbohydrates than did those

TABLE III

NITROGEN IN SOYBEANS GROWN IN SAND AND MANURE

MATERIAL	SAND			MANURE		
	NITROGEN			NITROGEN		
	SOLUBLE	INSOLUBLE	TOTAL	SOLUBLE	INSOLUBLE	TOTAL
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Leaves	0.13	0.80	0.93	0.09	0.78	0.87
Stems	0.40	0.24	0.64	0.42	0.22	0.64

FIG. 3. Root system of soybean grown on manure. Sparse nodule development.

grown in either soil (Wooster silt loam) or manure (3 parts of manure to 1 of Wooster silt loam). In general, the increase was due chiefly to easily hydrolyzable carbohydrates, cellulose, and lignin.

The increase in carbohydrates in the plants grown in the sand was not accompanied by a simultaneous decrease in nitrogen as has been found in the case of certain non-legumes. The high nitrogen content of these plants, however, was associated with the development of relatively large numbers of nodules on the roots of the plants.

The stems of the plants grown in the sand were comparatively tough and rigid and not inclined to lodge.

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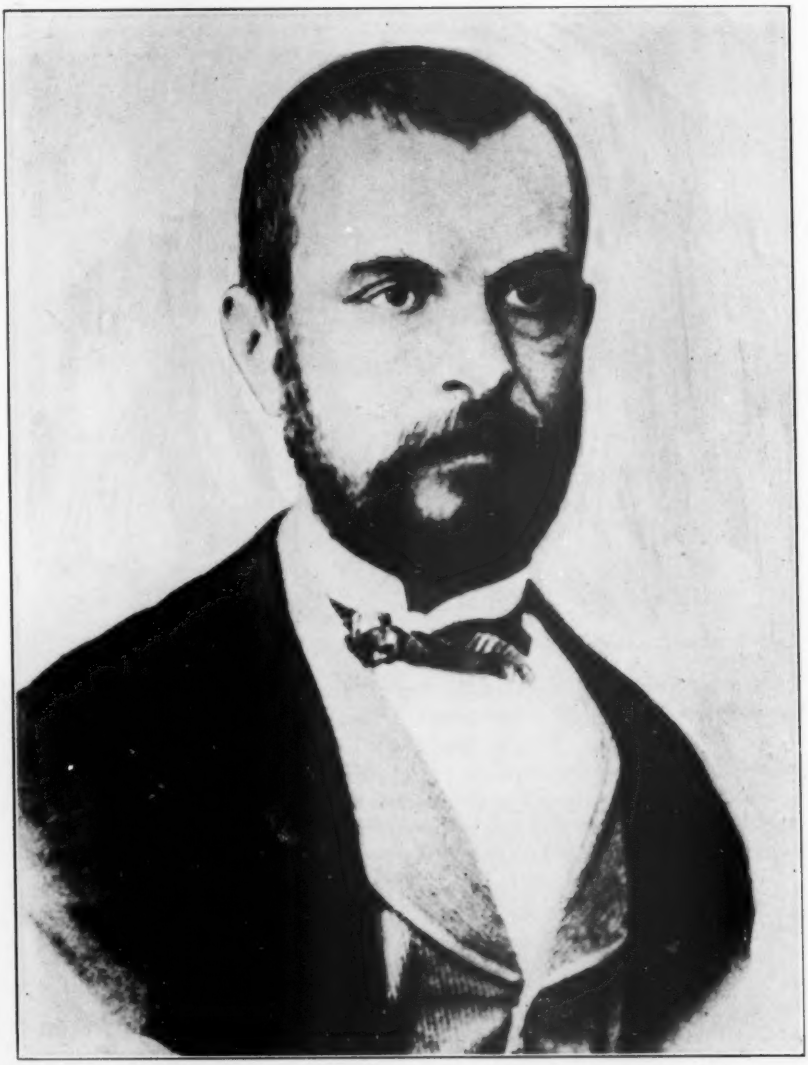

WILHELM HOFMEISTER
1824–1877

BRIEF PAPERS

WILHELM HOFMEISTER

FREDRICH WILHELM BENEDICT HOFMEISTER, the son of a proprietor of a music store, publisher, and book seller, was born at Leipzig, May 24, 1824. The early part of his life was spent as a clerk, with nature study as a hobby. Durng later years he was head of Botany Departments, first at the University of Heidelberg and later at the University of Tübingen. He assumed these positions without university preparation and with only experiences as a self-taught student of botany to recommend him. These experiences, and experiments, began shortly after he left school at fifteen years of age, and led to some of the most profound discoveries ever made in the field of botany. Most of his work was accomplished in the face of the necessity of providing for a large and growing family, and with the physical handicap of poor eyesight, for he was very near-sighted. He pursued the study of plants before and after hours of clerical work and at odd times.

Hofmeister's earlier education was obtained in a private school, the later, in the Leipzig Realschule, where the master taught in a manner that made the pupils think for themselves. He left this school at fifteen years of age to face the world. He early showed an inclination to study nature, largely due to the influence of his father, who was also interested in plants and maintained a small but rather varied, systematically arranged garden. At first he was more strongly inclined towards zoology, but finally decided upon botany. His first salaried position was one in a music store at Hamburg, where in his loneliness he took to roaming the fields. All the while he continued self-education by studying languages, music, physics, chemistry, algebra, geography, etc. Remaining a student to the end, ever trying to improve, he learned Greek in his fortieth year.

After two years at Hamburg he returned to Leipzig and entered into business with his father, where he had leisure time to study. Here he spent sixteen years and did most of the work for which he is famous. At nineteen years of age he had started many fundamental studies that placed him in the front rank of botanists. SCHLEIDEN'S Outlines of Scientific Botany awakened his interest in developmental history and microscopic research. He made field trips even outside of Germany.

HOFMEISTER was an excellent teacher for advanced students, rendering excellent service in technique and the preparation of materials, besides being a wonderful incentive to young botanists, and a drawing agency for advanced students. Among these at Heidelberg were: ASKENASY, ENGELMANN, KIENITZ-GERLOFF, N. J. C. MULLER, BOEHM, PFITZER, KRUTITSKY, ROSANOFF, TIMIRIASEV, WOLKOFF, and MILLARDET, and at Tübingen

ZACHARIAS and VON GOEBEL. His quarters were modest. He spent the whole day in the laboratory assisting students. He seemed to be truly indefatigable.

HOFMEISTER was a man of small stature, dark skinned, with vivacious eyes, the quick movements of a southern Frenchman, a very refined character and a kindly humor. He was exceedingly dextrous in the laboratory, near-sightedness assisting in making very good microscopic slides. Poor eyesight was a handicap in other respects, but he was too sensitive about his appearance to wear glasses. This condition frequently got him into humorous positions, as, for instance, greeting every woman he met on the street for fear of appearing to slight some one. His greetings were not mere perfunctory salutes. He was excellent company and possessed a remarkable memory and mind, one that had room for more than the details of his profession. The photograph, plate IX, shows him at the age of 43 years.

HOFMEISTER, in the happy years of a short life, without guiding assistance other than that of his father and occasional visits with botanists, did a monumental work and settled for all time, disputes in regard to certain phases of plant life. His interests and endeavors in plant research were wide and many.

CAMERARIUS in 1695 had shown that sexuality existed in plants but could not explain the fertilization process. KÖLREUTER experimented in crossing plants and demonstrated the presence of inheritance. The details of the process were lacking. In animals LEEUVENHOEK thought the spermatozoa produced the embryo and that the female matured it. SCHLEIDEN thought the tip of the pollen tube became the embryo in a similar manner. He fought stubbornly for this and because of his prestige and gift of argument, really obstructed progress. There were numerous theories of fertilization, of which the above are examples, but when twenty-five years of age, HOFMEISTER settled the question for all time. His technique was so marvelous that in *Oenothera* he could remove the pollen tube from the embryo sac without mutilating either. The publication in 1848 of this work in the *Botanische Zeitung,* entitled "Die Entstehung des Embryo der Phanerogams," is a marvel of direct exposition and is typical of his style. His drawings are numerous, detailed, and exact. This work won him an honorary degree of Ph.D. from the University of Rostock. He afterwards worked on nineteen families of plants. He had seen the egg sac with its egg apparatus and antipodals. His lack of university training was no longer a handicap. The Royal Saxon Society of Science at Leipzig admitted him to membership.

Turning to comparative morphology, HOFMEISTER endeavored to show a continuity through the plant kingdom but seemed to have no thought of the possibility of evolution. He found the function of the spermato-

zoids and archegonia in the liverworts and related it to the other cryptogams and phanerogams, and clearly demonstrated the alternation of generations. JULIUS SACHS gave him full credit for the establishment of the basis of phylogeny throughout the plant kingdom. HOFMEISTER saw and recognized the process of fertilization and the liberation of zygospores in the desmids and diatoms. He noted also many causal things about plant structure; distinguished between axial, leaf, and hair structures, and noted their relations. He recognized inheritance, variability, and the mutation of plants.

The controversy in regard to the origin of the cell was waxing hot, before his call to Heidelberg, with MIRBEL, VON MOHL, SCHLEIDEN, and NÄGELI furnishing most of the commotion. NÄGELI's concept is the one commonly accepted today, but HOFMEISTER made many contributions to the details. He saw nuclear division in an embryo and thought that one-half of the protoplasm collected around each daughter nucleus. In pollen grains he saw the nuclear membrane, and saw it and the nucleoli disappear before cell division, and possibly the cell plate and chromosomes. He recognized the importance of colloids and their functions in the physical properties of protoplasm, and studied the permeability of protoplasm. He saw that protoplasm was most abundant in young cells and that cell growth preceded cell division, which took place perpendicular to the direction of strongest growth. Many had considered plants as being composed of independent units or cells. HOFMEISTER considered the cells as being parts of a correlated structure. He anticipated and saw plasmodesmen, establishing that plants are made up of united protoplasmic structures. He said cell turgor was due to osmotic pressure of the cell contents and to water absorption.

HOFMEISTER's experimental physiological work was chiefly on the movement of sap. He noticed that bleeding took place when there was a reduction of evaporation and that it was not confined to woody plants. He found a sap pressure of 212 mm. of mercury with the poppy and 461 with *Digitalis*. He thought that the tension in the parenchymatous cells and the guttation of cell cavities forced soil water into the vessels. He discovered the periodicity of sap movement in vines and the negative pressure in stems. He observed the curvature of stems due to shock and their subsequent straightening out. He thought heliotropism was due to a lack of light on one side which caused greater elongation on that side. The geotropic response of roots he thought to be a passive response to gravity, the one big failure in his observations. He observed that a rise of temperature caused tulip flowers to open and a decrease caused them to close.

In his twenty-third year HOFMEISTER married the very refined daughter of a Leipzig manufacturer, who created for him an extremely happy and beautiful domestic life. His father had built a large house in Reudnitz,

outside of Leipzig, facetiously called by the members of the family and friends, the "Patriarchal Tent," where he and his children's families lived in perfect accord. It was a most happy community in which social relations functioned perfectly and made an ideal setting for Wilhelm's wonderful work. Here he spent sixteen of the happiest and most fruitful years of his life. HOFMEISTER was called to the University of Heidelberg as Professor of Botany in Ordinary, and Director of the Botanical Gardens at the age of thirty-nine. All went well until he moved to Heidelberg, where his wife contracted pulmonary ailments and died a few years later. This was a great blow to him and left him prostrate. Then his youngest daughter also contracted pulmonary ailments and died. This was a further severe shock to him and seriously interfered with his labors. A second daughter died soon afterward. His courage and best powers were gone. At this time dissention appeared in the faculty at Heidelberg. He did his utmost to avoid being drawn into it. On the death of HUGO VON MOHL at TÜBINGEN, he accepted the call to fill his place, but misfortune followed him. Three of his sons had died in infancy, and now the other two, one twenty-one and the other twenty-five, contracted pulmonary troubles, presumably tuberculosis, and were sent to southern France to recuperate. Here they died within a few months. He was unable to attend their funerals since the letter in regard to their condition did not reach him, and the laws of France required burial within twenty-four hours. His grief overwhelmed him. He became morose and dejected, with only an occasional return of his former self. HOFMEISTER was keenly interested in the unity of Germany, and during the Franco-Prussian war seemed to revive, but for only a brief period. With the death of his two sons he saw the passing of his name. He married the daughter of a physician at Lindenau, but there were no children of this marriage. The honor bestowed by the Dutch Society of Science afforded him his last pleasure. He became palsied and lost his speech, an indication of the blows which were to follow. He suffered a stroke of paralysis, from which he recovered enough to resume his lectures for a short time. A second stroke followed, and it was evident that he must retire from his post. Returning to Reudnitz in the fall of 1876, he seemed to revive, but soon suffered another stroke on January 5, from which he failed to recover, dying a week later, on January 12, 1877, in his fifty-third year. Thus closed a brilliant life that under more favorable conditions might have made many more contributions to botanical science.

One must marvel at the immense amount of work of high caliber that HOFMEISTER accomplished in his short life of fifty-three years under adverse conditions. We should especially remember him for explaining the process of fertilization and for laying the foundations of phylogeny.—A. H. LARSON, *University of Minnesota.*

USEFUL DEVICE FOR EVAPORATING ALCOHOL FROM PLANT EXTRACTS

(WITH ONE FIGURE)

Investigators who analyze plant material are always interested in methods which will save them time and labor in carrying out the numerous steps involved in most analytical procedures. In carbohydrate analyses, one of the steps which requires much time and is often troublesome is the removal of the alcohol after extraction with this solvent.

It has been the experience of plant chemists that the removal of alcohol must be complete if subsequent clearing of the solutions is to be satisfactory. Moreover, if certain so-called "biological methods" are to be employed in hydrolysis—as for example, the use of the enzyme emulsin in the determination of glucosides—the extracts must be free of alcohol, since this enzyme is easily inhibited even by low alcohol concentrations. But however thoroughly the alcohol needs to be removed, the process is troublesome and time-consuming. If the plant samples taken for analyses have been preserved in alcohol, instead of being oven-dried, the removal of alcohol becomes even more of a problem.

The method commonly used is to evaporate the alcohol on a steam or hot water bath, either with or without reduced pressure. If evaporation is conducted at atmospheric pressure several hours are generally required and the solutions, at least in the later stages of evaporation, are subjected for a considerable time to a temperature very close to 100° C. Thus, any advantage of using the low boiling solvent, alcohol, for extraction is lost by subjecting the extracted materials to the temperature of boiling water.

If reduced pressure is used the time required to free the extract of alcohol is somewhat shorter, although it is still quite long. The method has an advantage in that the alcohol is recovered and can be re-used. However, at the relatively low cost of commercial alcohol, it is very doubtful if this advantage offsets the numerous disadvantages of the method. The apparatus requires more or less careful attention to insure the proper intake of air to carry off the alcohol vapors and yet prevent splashing of the solutions which would result in a loss of solutes; the flasks occasionally collapse, due to outside pressure and the extract is lost; the rate of removal of alcohol usually does not take place uniformly in the various flasks, necessitating repeated attention from the analyst.

The device reported here is very simple and provides a rapid and satisfactory method of alcohol evaporation. It consists of an air pump driven by a 1/6 H. P. motor, and connected to a piece of 0.75 inch iron water pipe

about 8 feet long into which are fitted, at the proper intervals, 12 curved pieces of 3/16 inch copper tubing each about 7 inches long. The apparatus described accommodates twelve flasks, although for convenience of illustration, a battery of only six is shown in fig. 1.

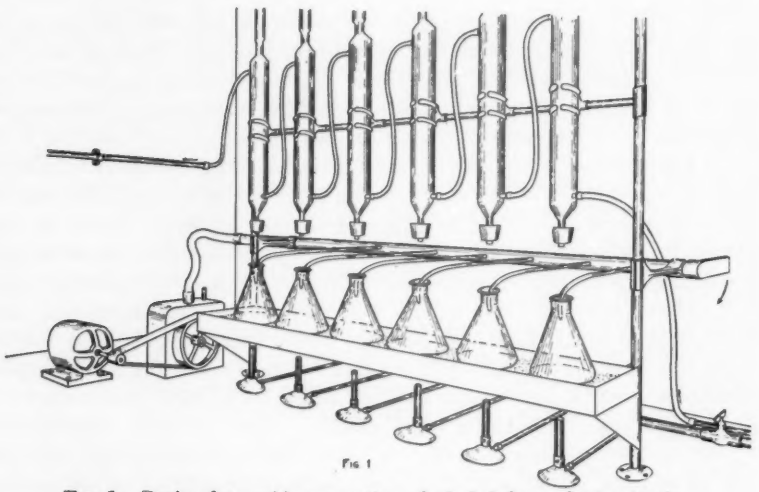

FIG. 1. Device for rapid evaporation of alcohol from plant extracts.

The pump used is a Crowell positive blower, delivering 13 cu. ft. of air per minute. This supply of air, when divided among twelve flasks, is sufficient for rapid evaporation. The pipe carrying the air is fastened in such a way to the back of the stand which holds the condensers that it can be turned in its clamps to swing the copper delivery tubes into the position shown, or back out of the way. A piece of rubber hose connects the pipe with the pump and permits the pipe to turn freely. The apertures of the copper tubes have been reduced by pinching the ends, thus regulating the amount and the force of the air stream from each. A more elaborate arrangement for regulating the air stream could doubtlessly be secured by special fittings for each copper delivery tube.

In order that the Soxhlet tubes can be inserted for extraction, the pipe is turned in its clamps to swing the delivery tubes back out of the way. After extraction all that is necessary is to remove the Soxhlet extractors and turn the pipe so that the delivery tubes enter the mouths of the extraction flasks. The pump is then started and evaporation proceeds at once.

The figure shows a sand bath heated by gas flames. An electric hot plate, of course, serves as well, but if gas is used it should be turned off

when evaporation is started so that the alcohol vapors driven out of the flasks cannot ignite. The residual heat of the sand bath (or hot plate, as the case may be) aids very materially in the evaporation, and yet is not sufficient at the time of complete evaporation to caramelize the carbohydrates in the flasks. This, however, is never a danger as long as the flow of air is maintained. In preliminary work with the apparatus, sugar solutions were evaporated to complete dryness with the gas heat on and yet no caramelization occurred because of the cooling effect of the rapid air stream. In actual work, the extra precaution of turning off the heat is always followed.

Before the pump is started the alcohol in the flasks is at its boiling temperature, but when the flow of air is begun the temperature drops quickly to approximately 35° C. (in the case of 95 per cent. alcohol) due to the cooling effect of rapid evaporation. Near the drying point the temperature may rise to 40° C. The time necessary for the evaporation of 100 cc. of 95 per cent. alcohol is from fifteen to twenty minutes, and even for 80 per cent. alcohol the time is considerably less than one hour. The method is thus seen to effect a rapid evaporation at a low temperature and with a simplicity of operation which requires no special care on the part of the analyst.

Apart from its usefulness in the removal of alcohol, which alone justifies its existence in this laboratory, the device is frequently found useful in various procedures where evaporation is necessary. Its description is presented here with the hope that other investigators can use it to advantage. —F. E. Gardner, *Department of Horticulture, University of Maryland, College Park, Maryland.*

NOTES

Seventh Annual Meeting.—The seventh annual meeting of the American Society of Plant Physiologists will be held in Cleveland, Ohio, Dec. 29, 1930–Jan. 3, 1931, in connection with the meeting of the American Association for the Advancement of Science. The headquarters of the Society will be at the Hollenden Hotel, along with the other botanical societies. The call for titles of papers was sent out some time ago, and the Secretary and Program Committee should have the support of everyone in making this a meeting of unusual value.

The annual dinner, Monday, December 29, will be featured again, it is hoped, by the announcement of the second Stephen Hales prize, and the annual award of the CHARLES REID BARNES Life Membership. Members should reserve this evening first, before making any other engagements for dinner programs, or evening lectures.

Cleveland is an interesting, progressive city, and can be depended upon to handle large meetings comfortably. It is centrally located for the northern states. A large and enthusiastic meeting is an inspiration to a greater research program for each individual participant. One cannot afford to miss the dynamic stimulation which comes from meeting one's contemporaries in intimate friendship.

Fifth International Botanical Congress.—The International Congress held at Cambridge, Aug. 16–23, 1930, was a great success. No larger gathering of botanists has ever taken place, and important steps were taken in connection with the work of taxonomists which will have a lasting effect upon botany.

The surroundings of the Congress were ideal, and about 1200 were registered as members, although the number actually in attendance was somewhat less, about 1000 delegates. The meetings were held in the buildings and laboratories of the Colleges of Cambridge University. Examination Hall and the Arts School were used for reception and social purposes during the entire period.

Plant Physiology had probably the largest single section of the Congress, and was credited with about 200 delegates. The next largest group, Ecology, was estimated at 150–200. The meetings of the Physiology Section were held in the central lecture hall on the first floor of the Botany School. The meetings were presided over by Dr. F. F. BLACKMAN, assisted by some of the numerous vice-presidents of the section. There were fourteen of these, as follows: W. L. BALLS, V. H. BLACKMAN, E. DEMOUSSY, H. H. DIXON, P. BOYSEN JENSEN, L. JOST, M. KORCZEWSKI, F. E. LLOYD, H. G. LUNDEGÅRDH, N. MAXIMOW, W. J. V. OSTERHOUT, W. RUHLAND, A.

URSPRUNG, and F. A. F. C. WENT. The recorder of the Section was WALTER STILES, and the secretary, G. E. BRIGGS. The vice-presidents were asked to occupy the front row of seats which formed part of a great circle in front of the demonstration table and the President's desk. This was done presumably to lend dignity and impressiveness to the occasion, and to insure that the front seats would be occupied. The vice-presidents discharged this double duty admirably.

The programs were originally outlined for six days, but owing to the absence of all Russian delegates, the papers assigned to Saturday were set back to Friday morning, leaving the final session vacant for more enjoyable purposes, as it was a day of leave-taking. The program on Aug. 18 was devoted to the problems of carbon metabolism, especially respiration, a field to which the Cambridge School, under Dr. BLACKMAN'S leadership, has given much attention in recent years. Two of the papers by RUHLAND and BENNETT-CLARK, dealt with the physiology of the organic acids which are related to the carbohydrates.

The following day was given to the consideration of permeability and osmotic behavior of plant membranes. This program was led by JOST'S interesting paper on protoplasmic permeability. Prof. PRÁT of Prague showed moving pictures of plasmolysis and swelling of cells, and other studies of permeability were presented by HÖFLER, IRWIN, ÚLEHLA, SHULL, and BRAUNER. The program emphasized the great complexity of permeability phenomena, and the impossibility of explaining it according to any single hypothesis.

The Wednesday program centered about mineral nutrition and growth of higher plants. LUNDEGÅRDH described a spectroscopic method of determining the quantity and kind of ions present in soil or plant solutions, and other papers dealt with phosphoric acid concentrations and growth of corn, mineral nutrition of barley, selective absorption of potassium, and absorption of minerals from insoluble compounds. At 4:30 P. M. Prof. ÚLEHLA presented a splendid moving picture film on plant development at the Cinema Theater. It showed autonomic and tropic movements of seedlings, inflorescences, etc., and was much appreciated.

On Thursday, certain methods of investigating protoplasmic organization were discussed. Bioelectric potentials, hormones, X-ray studies, *intra vitam* staining, and regeneration of woody cuttings were all included under this broad heading. The final sessions took up growth and development of higher plants. V. H. BLACKMAN'S high tension electric discharge experiments on barley growth were discussed, and such topics as nutrient ions and enzyme activity, translocation of salts and metabolites, root behavior of cotton, the metabolism of xanthine derivatives, alkaloids, and urea in plants,

and the structure and function of *Utricularia* traps, filled out an interesting and valuable program.

Too much cannot be said in regard to the cordial spirit of the meetings and the social arrangements of the Congress as a whole. In the general plenary meetings, the Congress has, let us hope, found a permanent solution to the species muddle. A compromise was reached, in which the spirit of give and take were approximately equal. All past work in languages other than Latin are validated, but from 1932 on, descriptions are to be in Latin. Concessions were made to American workers in regard to types. As the rules regarding descriptions were passed unanimously, one should have the right to hope that all taxonomists will obey the rules, or leave the description of species to those who will. The settlement of these controversial questions in an amicable manner by the Congress is a great step forward.

The Congress also voted to accept an invitation sponsored by Dr. F. A. F. C. WENT, of Utrecht, to hold the next Congress in Holland in 1935. It is not at all likely that the meetings would be held at Utrecht, but possibly at Amsterdam or Rotterdam, where accommodations would be more adequate.

Membership.—Plant physiologists, or those interested in the literature of plant physiology who desire membership in the American Society of Plant Physiologists, can make application for membership directly to the Secretary-Treasurer, Dr. WRIGHT A. GARDNER, Department of Botany, Alabama Polytechnic Institute, Auburn, Alabama. The membership has become international in character, and plant physiologists in foreign lands are also invited to make use of the privilege of direct application to the Secretary. Members may also send in the names of friends and colleagues who have expressed a desire to become members. The rapid growth of the Society during the last year has now given to PLANT PHYSIOLOGY as large a circulation as the old well established journals. It reaches more plant physiologists than any other botanical journal.

Portraits.—There are now eight portraits in the gallery which is being made available by PLANT PHYSIOLOGY to the members of the Society. They are purchasable at 12 cents each, postage paid. During 1931 it is hoped to continue these portraits, and to add portraits of contemporary plant physiologists chosen from among those who have attained the highest distinction in this field, especially in countries other than the United States.

International Address List.—The second edition of the International Address List was sent out to the members last spring. Some omissions will be noted on looking it over carefully. It does not need to be said that these

omissions are unintentional. Whenever any one finds a name missing, it should be reported, so that future editions can be corrected. To help cover the cost of this bulletin, the Secretary omitted the regular membership bulletin, substituting a mimeographed supplemental list of new members added during the previous year. The International Address List is so valuable that a new edition should be printed every five years, even if we have to forego the annual membership list once in five years.

Errata.—The errors found in the earlier numbers of Vol. 5 of PLANT PHYSIOLOGY are placed at the end of the table of contents in this issue. If the corrections are inserted at the places where the errors occur, it may prevent misunderstanding and misinterpretations later on. Members are urged to correct all of their files in this way. Assistance on the part of authors and readers in detecting errors is invited at all times.

Celebration at Rutgers University.—The Agricultural Experiment Station of New Jersey celebrated its fiftieth anniversary on October 8–9, 1930. Nearly a thousand visitors participated in this happy occasion. Open house was held by the laboratories from 10:00 A. M. to 2:00 P. M., after which there were a number of addresses, and the unveiling of a memorial tablet to the first two directors of the Station, Dr. GEORGE H. COOK, and Dr. E. B. VOORHEES. In the evening a dinner was held in Cooper Hall, with about 475 in attendance. Dr. J. G. LIPMAN, the present Director, was the toastmaster, and Sir JOHN RUSSELL of Rothamsted, and Dr. S. ORLA-JENSEN of Copenhagen were among the after-dinner speakers.

On the second day, a meeting was held in the Elizabeth Rodman Voorhees chapel, at which honorary degrees were conferred upon Drs. C. F. MARBUT, LAFAYETTE B. MENDEL, THEOBALD SMITH, Sir JOHN RUSSELL, S. ORLA-JENSEN, L. O. HOWARD, and CHESTER LATHROP PACK. Following the conferring of the degrees, a luncheon was held for official delegates from other institutions as guests of the Experiment Station staff.

The American Society of Plant Physiologists was represented at the celebration by Dr. JOHN W. SHIVE, who was appointed as the Society's delegate by President H. R. KRAYBILL. The New Jersey Station has an enviable record for aggressive research, and deserves a high place in the history of Experiment Station development in the United States. It has the good wishes of every one of its friends for a greater second half century.

Physical Measurements.—As an aid to the Committee on Physical Methods of the American Society of Plant Physiologists, the Leeds and Northrup Co., 4901 Stenton Ave., Philadelphia, has prepared a special

bulletin. This bulletin was prepared by Mr. MELCHER, and deals with measurements of hydrogen-ion concentrations, electrolytic conductivity, galvanometers, temperature measurements, measurements of radiant energy, humidity measurements, and gas analysis. The bulletin explains the usefulness of various types of instruments, and in case they are not handled by Leeds and Northrup Co., tells where they may be obtained.

They have also reprinted an article by Dr. C. Z. ROSECRANS on "The present state of apparatus for hydrogen ion measurements" from the January, 1930, issue of the Journal of the American Water Works Association. Copies of the special bulletin and of the ROSECRANS reprint may be obtained from Leeds and Northrup Co. on request, by any of our members. The Leeds and Northrup Co. Notebook no. 3 is a valuable text on the subject of hydrogen-ion determinations.

Physiology and Biochemistry of Bacteria.—Volumes 2 and 3 of this work by BUCHANAN and FULMER were mentioned briefly in the July number of PLANT PHYSIOLOGY. Volume 2 is on "The effects of environment upon microorganisms," and volume 3, "The effects of microorganisms upon environment, fermentations and other changes produced."

The authors must have a keen sense of humor to mention in the preface to volume 2 that the treatise is "intended to serve merely as an introduction to the subject." It is hoped that they continue the style and extensiveness of treatment for a complete and exhaustive discussion of Bacteriology, if more is to be given in the present state of knowledge of the subject.

Volume 2 is a thorough presentation of all phases of environmental effects on bacteria, with the physiology and biochemistry fundamental thereto. Students who are able to absorb even a fair portion of the information presented will be well trained in physiology, and biochemistry, as well as in bacteriology. The discussion of the effects of temperature on vital processes in volume 2 is especially good. The discussion of the effects of microorganisms in producing chemical changes in the environment in volume 3 is also excellent. One could not expect books of this kind to be entirely free of errors, but the authors have used an interesting style, the work is carefully compiled, and it is well printed. The bibliographies are quite complete for the subjects covered. The Williams and Wilkins Co., Baltimore, Maryland. Price, $7.50 each volume.

Plant Physiology.—The COULTER, BARNES, COWLES Text Book of Botany is appearing in a new three volume edition, each part paged independently. Volume two, Physiology, is now available, and can be purchased for $1.80 from the American Book Co., 88 Lexington Ave., New York. This volume was revised and enlarged by Prof. C. A. SHULL, of

Chicago. A large number of citations accompany the text, and literature references at the close of each chapter provide suggestions for much collateral reading.

Self Sterility and Hybrid Sterility.—From the press of Julius Springer, Berlin, we have received a copy of volume 21 of the Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere. It is entitled "Selbststerilität und Kreuzungssterilität im Pflanzenreich und Tierreich," and is written by Dr. FRIEDERICH BRIEGER, of the University of Berlin. Following a brief introduction, the work is presented in three sections: A. Parasterility of the higher plants; B. Parasterility of the metazoa; and C. Parasterility of thallophytes and protista, A general discussion of the meaning of self sterility, problems and theories of inbreeding degeneration, and sexuality and parasterility concludes the work. A bibliography of about 700 titles indicates the thoroughness with which BRIEGER has covered the ground. Including the index, the book contains 395 pages. The price of the monograph is 32 RM unbound, and 33.8 RM bound in cloth.

Chemistry of Protoplasm.—The fourth volume of Protoplasmamonographien has been published by Gebrüder Borntraeger of Berlin. It is by Prof. ALEXANDER KIESEL, of the University of Moskau, and is entitled "Chemie des Protoplasmas." There are eight chapters: Protoplasm as a whole; cytoplasm and the chemistry of its morphological structure; cytoplasm and its chemical substances; the nucleus and its constituents; the resting nucleus and division; the chemical substances of the nucleus; nonnucleate cells; and plasmodium of the Myxomycetes as a protoplasmic material. A bibliography of 568 titles is included. A book of this kind impresses the reader in two directions—with how much, and yet how little —we really know about the chemistry of the fundamental living machinery of animals and plants. The price of this monograph is 20 RM, and orders should be addressed to the publishers, Berlin W 35, Schöneberger Ufer 12a.

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